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# RECURRENT ABDOMINAL PAIN IN CHILDREN – the concept, aetiology, diagnostics and prognosis

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# RECURRENT ABDOMINAL PAIN IN CHILDREN
## – the concept, aetiology, diagnostics and prognosis
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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Han klämde lite på honom och lyssnade på hans hjärta. Det var ju inget fel, det visste han. Hjärtat pumpade. Lungorna höjde och sänkte bröstet. Men han tyckte att nån borde röra vid honom.*

ur *Händelser vid vatten* av *Kerstin Ekman*

.

To my parents, Karin and Åke

* He touched him a little and listened to his heart. Obviously, nothing was wrong, he knew that. The heart was working. The lungs raised and lowered the chest. But he thought that someone should touch him.

# ABSTRACT

**Background:** Recurrent abdominal pain (RAP) due to an abdominal pain-related functional gastrointestinal disorder (ap-FGID) is one of the most common disorders of childhood and it accounts for much suffering and many healthcare efforts. Both school absence and parental work absence can be considerable when young children are affected. The aetiology of ap-FGID is regarded multifactorial, where the bidirectional gut-brain axis is thought to have a central role, involving gut microflora and psychosocial events. The biopsychosocial model seems superior to standard medical care when treating children with ap-FGID. The current terminology adheres to the symptom-based Rome criteria of different ap-FGIDs, most recently updated 2016 in Rome IV. There are scarce evaluations of the accuracy of the paediatric Rome diagnostic criteria and suggested alarm symptoms in diagnosing ap-FGID.

**Methods**: This thesis was founded on four studies, based on two population-based birth cohorts and one clinical study.

In study I, 258 children 4-17 years old seeking care for gastrointestinal complaints were evaluated. Validated Rome III based patient-administered symptom questionnaires were filled in at the initial visit, blinded to the clinician. One year after inclusion a reference diagnose was set through review of electronic records. The sensitivity and specificity of the Rome III criteria for ap-FGID combined with selected alarm symptoms and/or laboratory tests were explored in four-field tables.

Study II was based on a population-based birth cohort, BAMSE (n=4089). The association between antibiotic use and RAP at 12 years was explored. Information on early childhood antibiotic use during the first two years of life was based on parental questionnaires. Antibiotic use during the three years before gastrointestinal evaluation at 12 years of age was derived from the Swedish prescribed drug register. RAP was defined as monthly abdominal pain according to questionnaire self-report.

Study III was performed in a subset of the lifestyle birth cohort ALADDIN (n=470). Children growing up in families of different lifestyles were explored regarding ap-FGID at five years of age. The three different, predefined family lifestyles were anthroposophic, partly anthroposophic and non-anthroposophic and classified through parental questionnaires. The Rome III-defined ap-FGIDs irritable bowel syndrome, functional dyspepsia and functional abdominal pain were assessed at five years through questionnaires and telephone interviews.

Study IV was based on the BAMSE cohort. RAP was measured on three time points: in early childhood, containing pooled data from 12 and 24 months, at 12 years and at 16 years. In early childhood RAP was defined as parental report of abdominal pain during the previous 6 and 12 months respectively. Questionnaire data at 12 and 16 years were self-reported, and RAP defined as weekly abdominal pain. At 16 years the questionnaires also allowed for definitions according to Rome III for ap-FGID, irritable bowel syndrome, functional dyspepsia and functional abdominal pain. Binominal generalised linear model with log link

was used to assess the association expressed as relative risk, between RAP in early childhood or at 12 years and RAP at 12 and 16 years, and any ap-FGID and irritable bowel syndrome at 16 years.

**Results:** In study I we found that Rome III criteria for ap-FGID in combination with absence of any alarm symptoms had high specificity (0.90) but very low sensitivity (0.15) in diagnosing children with ap-FGID. Alarm symptoms were equally common (81 % in total cohort) regardless of the cause of gastrointestinal complaints.

In study II we found no evidence that antibiotic use was associated with monthly RAP at 12 years of age.

The lifestyle study (III) revealed an increased risk of ap-FGID at five years in children growing up in families with a partly anthroposophic lifestyle, adjusted Odds Ratio 2.6 (95 % confidence interval: 1.1-5.9). No specific factor of the anthroposophic lifestyle could be identified as bearing this increased risk.

The prognosis study (IV) showed that most children with RAP in early childhood were not affected at 12 years of age, adjusted Relative Risk (RR) 1.7 (95 % CI: 0.9-3.0). Most children with RAP at 12 years of age were not affected at 16 years but had an increased risk of RAP at 16 years, adjusted RR 2.1 (95 % CI: 1.7-2.7), and of ap-FGID at 16 years of age, adjusted RR 2.5 (95 % CI: 1.8-3.4). In the cohort 33 % of children had RAP at least once between one and 16 years of age.

**Conclusion**: The Rome III criteria combined with absent alarm symptoms classified very few children with ap-FGID. When criteria were fulfilled, and alarm symptoms negative in children with gastrointestinal complaints, the risk of an organic disease was low. Antibiotic use was not a major risk factor for RAP. Children in families with distinct differences in family lifestyle had a two-fold increased risk of ap-FGID at five years, but mechanisms are unclear. We speculate in differences in childhood coping development. The prognosis in RAP was benign, with most children growing out of their symptoms. The increased risk of persistent RAP between 12 and 16 years that was found, can be useful in guiding clinicians on how to follow up children with ap-FGID.

# LIST OF SCIENTIFIC PAPERS

I. **UUSIJÄRVI A**, Olén O, Malmborg P, Eriksson M, Grimheden P, Arnell, H. *Combining Rome III criteria with alarm symptoms provides high specificity but low sensitivity for functional gastrointestinal disorders in children*. Acta Paediatr. 2018 Feb 27 [Epub ahead of print]

II. **UUSIJÄRVI A**, Bergstrom A, Simrén M, Ludvigsson J F, Kull I, Wickman M, Alm J, Olén O. *Use of antibiotics in infancy and childhood and risk of recurrent abdominal pain - a Swedish birth cohort study*. Neurogastroenterol Motil. 2014 June; 26(6):841-50.

III. **UUSIJÄRVI A**, Alm J, Lindblad F, Olén O. *Irritable bowel syndrome and functional abdominal pain in five-year-old children are related to lifestyle*. Acta Paediatr. 2016 Aug; 105(8):971-8.

IV. Sjölund J, **UUSIJÄRVI A**, Törnkvist N, Kull I, Bergström A, Alm J, Törnblom H, Olén O, Simrén M. *Recurrent abdominal pain from birth to adolescence - a prospective Swedish birth cohort study*. In manuscript

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ap-FGID | abdominal pain-related functional gastrointestinal disorders, Rome III |
| CBT | cognitive behavioural treatment |
| CD | celiac disease |
| CI | confidence interval |
| FAP (-NOS) | functional abdominal pain (not otherwise specified) |
| FAPD | functional abdominal pain disorders, Rome IV |
| FD | functional dyspepsia |
| FGID | functional gastrointestinal disorders |
| FMT | funktionell mag-tarmsjukdom |
| FODMAP | fermentable oligo-di-mono-saccharides and polyols |
| IBD | inflammatory bowel disease |
| IBS | irritable bowel syndrome |
| IgA-TGA ab | serum Immunoglobulin-A-transglutaminase antibodies |
| OGID | organic gastrointestinal disease |
| RAP | recurrent abdominal pain |

# 1 INTRODUCTION

Recurrent abdominal pain (RAP) is one of the most common medical complaints during childhood and adolescence [1-3]. RAP is prevalent in several clinical settings and often accompanied by substantial family worries and parental work absence [4, 5]. Some children and adolescents are exposed to a lot of costly and invasive medical investigations with typically scarce findings and small benefit for the child [6, 7]. If a specific diagnosis of the pain cannot be established it may capture the child and family in repeated and new investigations, with possible negative effects on the prognosis of pain, illness behaviour and disability [8].

The clinical presentation of RAP shows large variations. The frequency and duration of the pain attacks and the length of periods with pain are highly variable. There are also large differences concerning suffering and impact on everyday life such as school absence.

The aetiology of childhood RAP is also varied, and the cause is considered multifactorial. The currently most customary approach to functional gastrointestinal disorders is the "biopsychosocial model" [9]. This model implies that symptoms are a result of the bidirectional interaction between physiological processes and psychosocial influences [10]. Treatment according to the biopsychosocial model relies on validation, family education and reassessment, with the aim of symptom reduction and return to normal activities [9].

# 2 BACKGROUND

## 2.1 DEFINITIONS

The large variety of symptoms and aetiological factors related to childhood recurrent abdominal pain is reflected in many different definitions intending to describe the phenomenon. The concept of RAP has already been introduced and will be described more in detail below. Moreover, the Rome criteria will be described in detail.

### 2.1.1 Recurrent abdominal pain (RAP)

The term RAP is today commonly used as a description and not as a diagnosis, a childhood condition with long-lasting, intermittent or constant abdominal pain (Table 1). Apley and Naish, two English paediatricians, published a classical, often cited study in 1958 on 1000 children with 'recurrent abdominal pain', thereby introducing this concept into the scientific literature [11]. RAP was defined as ≥3 bouts of abdominal pain severe enough to interfere with activities, appearing in a period of ≥3 months. The cause of pain was not considered, i.e. functional and organic RAP were not separated, which is a major difference between their definition and later terminology according to the Rome foundation. In 1999, von Baeyer proposed a two-stage approach to the classification of children with RAP, where the second step referred to the cause of pain [12]. The use of the word RAP is prevalent in literature and scientific papers, but it does not always adhere strictly to the Apley & Naish definition from 1958. Approximately 90 % of paediatric cases with RAP seem to have a mainly functional background [3]. Examples of other causes of RAP in childhood are constipation, cow's milk protein intolerance, celiac disease (CD), esophagitis, inflammatory bowel disease (IBD), endometriosis (girls), duodenal ulcer and lactose intolerance.

Health care visits for paediatric acute abdominal pain are common in both primary care and emergency care settings [13]. Acute abdominal pain is also frequently caused by an abdominal pain-related functional gastrointestinal disorder (ap-FGID) but a number of other diagnoses should be considered. In a large academic primary care population of children ≥4 years of age, acute and chronic constipation was the most common cause of acute abdominal pain [14]. In the same study, a surgical cause was present in 2 % of subjects and in 19 % the cause for the acute abdominal pain remained unknown [14]. The differential diagnoses of acute abdominal pain in children varies greatly with the child's age and can be described as surgical, nonsurgical or extra-abdominal [15]. Examples of surgical causes are acute appendicitis and ovarian or testicular torsion. Non-surgical causes can be gastroenteritis, constipation, urinary tract infection or Henoch-Schönlein purpura. Extra-abdominal reasons for acute abdominal pain in children can be pneumonia, otitis media and diabetes mellitus [15].

| Table 1. Distinction between RAP and ap-FGID in thesis | |
|---|---|
| **RAP** | childhood condition of long-lasting, intermittent or constant abdominal pain, regardless of the cause but often medically un-explained |
| **ap-FGID** | scientific description of non-organic recurrent abdominal pain, that is pain of functional origin, according to Rome III criteria |

### 2.1.2 The Rome criteria

The Rome criteria are established, international classifications of both children and adults with functional gastrointestinal disorders (FGID) [16-18]. These criteria are intended for clinical as well as scientific purposes. The Rome foundation is an international, non-profit organisation with the goal to improve the lives of people with FGIDs, through research and education (https://theromefoundation.org/).

The Rome foundation has designed detailed questionnaires to enable descriptions and classifications of the different disorders, based on patients' symptom descriptions. There is a special, updated version for children, the Rome IV Diagnostic Questionnaire on Paediatric Functional Gastrointestinal Disorders (R4PDQ). The previous Rome III Questionnaire on Paediatric Gastrointestinal Symptoms (QPGS-RIII) has been translated to several languages and validated in different settings [19, 20].

At present, most research on RAP is based on the Rome criteria, that are regularly updated (Table 2). The Rome I criteria were defined for adults only. The first version of paediatric Rome criteria, Rome II, was published in 1999, using classifications according to main complaints, not according to the affected organ system [16]. The definitions of the different versions of the Rome criteria are presented in Table 2 which also, for comparisons, presents the Apley & Naish definition of RAP. The required symptom duration for ap-FGIDs in the Rome II-version was *three months* or more, except for abdominal migraine. In the updated Rome III-version this requirement was changed to a pain frequency of once weekly for *two months* or more [17, 20]. FGID can be divided in abdominal pain-related conditions (ap-FGID), and conditions where pain is not dominating. The ap-FGIDs are functional dyspepsia (FD), irritable bowel syndrome (IBS), functional abdominal pain (FAP) and abdominal migraine. In Rome III, all four have the additional diagnostic criteria of "no evidence of an inflammatory, anatomic, metabolic, or neoplastic process considered that explains the subject's symptoms".

**Table 2. CLASSIFICATION OF RECURRENT ABDOMINAL PAIN DISORDERS**

| Apley/Naish[11] 1958 | Rome II[16] 1999 | Rome III[17] 2006 | Rome IV[18] 2016 |
|---|---|---|---|
| **RAP: recurrent abdominal pain** | | **ap-FGID: abdominal pain-related functional gastrointestinal disorders** | **FAPD: functional abdominal pain disorders** |
| 1. ≥3 episodes of abdominal pain | 1. Persistent/recurrent pain/discomfort ≥12 weeks | 1. Persistent/recurrent pain/discomfort ≥once every week | 1. Symptoms of pain/postprandial fullness/early satiation ≥4 days/ month |
| 2. During a period of ≥3 months | 2. Within the preceding 12 months | 2. ≥2 months before diagnosis | 2. ≥2 months before diagnosis |
| 3. Pain severe enough to affect activities | 3. No evidence of an organic disease | 3. No evidence of an inflammatory, anatomic, metabolic, or neoplastic process that explain the symptoms | 3. After appropriate medical evaluation, the symptoms cannot be attributed to another medical condition |
| **Phenotypes:** | FD, IBS, FAP | FD, IBS, FAP, FAPS | FD, IBS, FAP-NOS |

FD, functional dyspepsia; FAP, functional abdominal pain; FAP-NOS, functional abdominal pain-not otherwise specified; FAPS, functional abdominal pain syndrome; IBS, irritable bowel syndrome

FD is defined as persistent/recurrent pain or discomfort centred in the upper abdomen, not associated with defecation or a change in stool frequency or stool form. IBS is defined as abdominal discomfort or pain associated with two or more of the following symptoms: improvement with defecation, onset of a change in stool frequency and/or onset of change in form of stool. FAP is defined as episodic or continuous abdominal pain and insufficient criteria for other FGID. FD, IBS and FAP require that criteria are fulfilled at least once per week for at least two months before diagnosis. Abdominal migraine, not part of the thesis, is

defined as paroxysmal episodes of intense, acute periumbilical pain that interferes with normal activities, lasting for one hour or more. It has a required occurrence of two or more episodes in the preceding 12 months, intervened by periods of usual health. The pain is associated with two or more of: anorexia, nausea, vomiting, headache, photophobia and pallor (Table 3).

The studies of this thesis were conceived before the introduction of Rome IV criteria in 2016, and therefore the terminology of Rome III will be used consistently. Out of general interest these up-dated criteria will be presented briefly below. The paediatric Rome IV criteria are divided in three parts: functional nausea and vomiting disorders, functional abdominal pain disorders (FAPD) and functional defecation disorders. The Rome III concept of ap-FGID was replaced by Functional Abdominal Pain Disorders (FAPD) [18]. A new term, "functional abdominal pain−not otherwise specified" (FAP-NOS) was introduced to describe children with recurrent abdominal pain that is neither IBS, nor Functional dyspepsia (FD) nor abdominal migraine. FAP-NOS, IBS and FD all have in common that "*abdominal pain must appear at least four times/month during two months"* prior to diagnosis, in addition to the statement that "*after appropriate evaluation, the symptoms cannot be fully explained by another medical condition"*. A modification of the words that describe the abdominal sensation was adopted, when *abdominal discomfort* in IBS and FAP of Rome III was replaced by *abdominal pain* in Rome IV. The wordings postprandial fullness and early satiation, not obligate abdominal pain, are used to describe FD in Rome IV.

The word "functional" in FGID is unspecific but relates to symptoms supposedly caused by the normal "function" of the gastro-intestinal tract. Apparently, the term has been under debate and with the Rome IV criteria in 2016, a new term has been established in the adult criteria: Disorders of the Gut Brain Interaction (DGBI)[21, 22]. With this expression, which will be described in detail in 2.2.2. Gut-brain axis, the Rome foundation incorporates the plausible aetiology of the disorders within the Rome terminology.

### 2.1.3 A positive diagnosis

The adult gastroenterologist Manning stated that a more careful history taking could prevent unnecessary investigations and increase diagnostic confidence in patients with RAP. In 1978 he suggested that irritable bowel syndrome (IBS) should be regarded as a positive diagnosis, based on four selected criteria: abdominal distension, pain relief with bowel action, more frequent stools with the onset of pain and looser stools with the onset of pain. The presence of three or four of these criteria in the adults of the study predicted IBS with a sensitivity of 62 % and a specificity of 85 % [23]. In the Rome III statement, the precursor of Rome IV, the additional criteria to symptom descriptions was that there should be "no evidence of organic disease". In Rome IV, this statement was modified. It was now claimed that an "appropriate medical evaluation" should be made after the symptom description, without pointing out certain tests [18]. So, in line with Manning's thoughts about a careful history taking, the Rome committee of 2016 seems to emphasise that functional diagnoses are clinical, and not dependent on tests and investigations.

## Table 3. ROME III ABDOMINAL PAIN-RELATED FUNCTIONAL GASTROINTESTINAL DISORDERS

**FUNCTIONAL DYSPEPSIA (FD)**

1. Persistent or recurrent pain or discomfort centred in the upper abdomen (above the umbilicus)

2. Not relieved by defecation or associated with the onset of a change in stool frequency or stool form

**IRRITABLE BOWEL SYNDROME (IBS)**

1. Abdominal discomfort or pain associated with two or more of the following at least 25 % of the time:

a. Improved with defecation

b. Onset associated with a change in frequency of stool

c. Onset associated with a change in form (appearance) of stool

**FUNCTIONAL ABDOMINAL PAIN (FAP)**

1. Episodic or continuous abdominal pain

2. Insufficient criteria for other FGID

**Obligate criteria in FD, IBS and FAP**:

- Criteria fulfilled at least once per week for at least two months before diagnosis
- All of the numbered criteria must be fulfilled
- No evidence of an inflammatory, anatomic, metabolic, or neoplastic process that explains the subject's symptoms

**ABDOMINAL MIGRAINE (not investigated in thesis)**

*Criteria fulfilled two or more times in the preceding 12 months:*
1. Paroxysmal episodes of intense, acute periumbilical pain that lasts for one hour or more
2. Intervening periods of usual health lasting weeks to months
3. The pain interferes with normal activities
4. The pain is associated with two or more of the following: anorexia, nausea, vomiting, headache, photophobia, pallor

*Source: Gastroenterology. 2016 Feb 15. pii: S0016-5085(16)00181-5. doi: 10.1053/j.gastro.2016.02.015.*

### 2.1.4 Alarm symptoms

The Rome IV statement that after "appropriate evaluation, the symptoms cannot be fully explained by another medical condition" is not very helpful in providing the paediatrician with instructions on what investigations should be performed in the child with RAP. One complementary diagnostic approach is the use of so called alarm symptoms, also called red flags [1, 18]. These are signs, symptoms and pathologic laboratory tests that, when present, decreases the probability of a functional diagnosis (Table 4). The concept of alarm symptoms is poorly defined, and definitions vary between publications and with patient's age. In adult gastroenterology efforts have been made to validate alarm symptoms for different definitions of FGID [24]. The alarm symptom *antibiotic use* appears in adult but not in paediatric studies. The number of alarm symptoms registered varies from 4 to 10 between studies. In most studies alarm symptoms are dichotomous ("yes" or "no"), but occasionally the symptom should have been present in ≥ 25 % of the pain attacks. The accuracy of each method depends not only on the questionnaire/definition used, but also on the setting and whether subjects with FGID are compared with healthy controls or subjects with organic disease. Accordingly, the prevalence of alarm symptoms shows great variations across studies [25-28].

## 2.2 AETIOLOGY

Most evidence suggests a multifactorial aetiology of paediatric FGID and ap-FGID. The amount of research on risk factors for ap-FGID is larger in adults than in children and results are often contradictive [29]. The biopsychosocial model has dominated research on childhood ap-FGID in the last decade [30]. A biopsychosocial perspective refers to the interaction between biologic and/or physiologic events and psychosocial factors, through the gut-brain axis, which will be discussed below [31-34]. Some specific childhood medical events like asphyxia, perinatal airway suction, pyloric stenosis, hypersensitivity to cow's milk protein and Henoch-Schönlein purpura have all been linked to FGID and ap-FGID in single studies [35-37]. However, not all factors shown to be associated with ap-FGID are possible to study with a prospective design. Thus, the cause and effect relationship is difficult to evaluate.

The aetiological factors that will be discussed below are: genetic predisposition, microbiota and gut-brain axis, inflammation, visceral hypersensitivity and motility, lifestyle and coping style, food, psychosocial factors and stress, exposure to adversities.

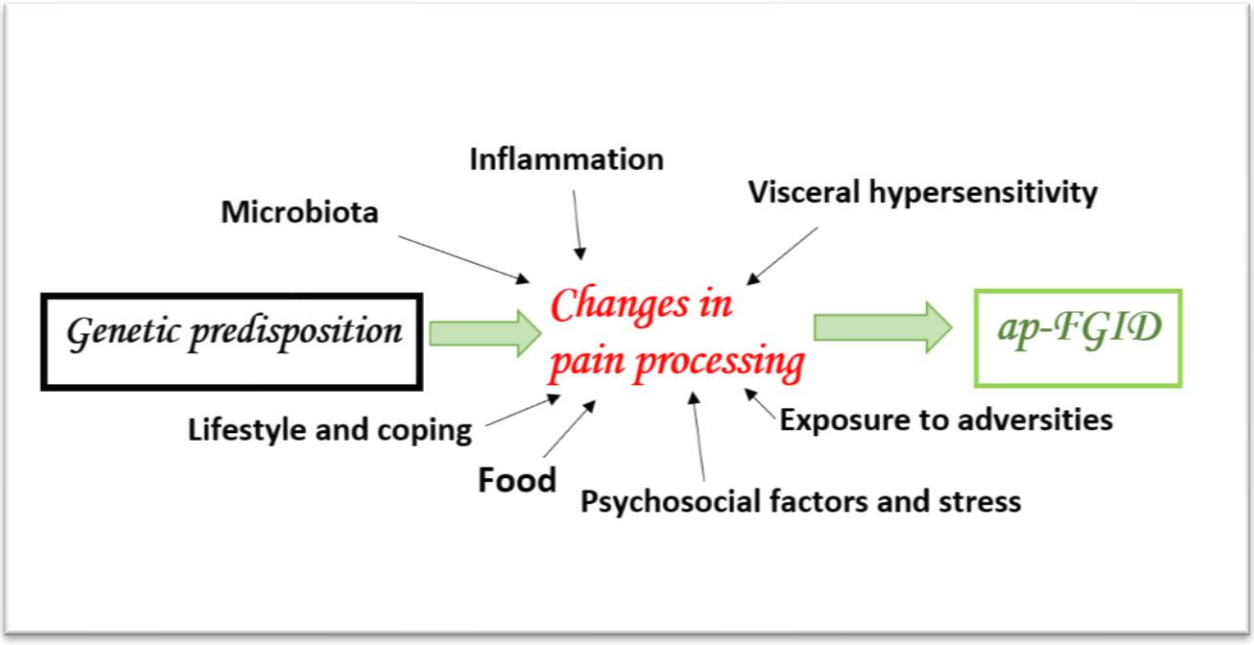


Figure 1. Pathophysiology of functional abdominal pain disorders. Modified after *Gastroenterology 2016;150:1456–1468*

### 2.2.1 Genetic predisposition

Visceral pain in relation to genetics has been described, indicating genetic variations in ion channels, barrier function and neurotransmitters in IBS patients [38]. A Norwegian study supported a genetic component in IBS, with monozygotic twins having 17 % concordance rate in IBS. In the same study it was also reported that having a parent with IBS was an independent predictor of IBS, stronger than having a twin with IBS. Thus, social learning had an equal or stronger influence than the genetics [39]. In a recent study on Rome IV diagnostic criteria, the mothers of 4-18-year olds completed online questionnaires. Children whose parents qualified for a FGID were more likely to qualify for a FGID themselves [40]. In a study from the BAMSE birth cohort, (not part of this thesis) a genetic variation at the NPSR1 locus,

which encodes the receptor for neuropeptide S (NPS), was associated to RAP in 12-year-old children [41]. The possible mechanisms of this genetic variation may act through increased expression of neuropeptides, thus modulating gut sensory and motor function.

### 2.2.2 Microbiota and the gut-brain axis

The intestinal canal is sterile upon birth but is rapidly colonised during the first year [42, 43]. The developing childhood microbiota has the potential to modulate the immune system and to ensure tolerance to foods [44]. Antibiotic treatment, diet and mode of delivery influence the gut microflora (Figure 2) [43, 45]. A bacterial gastroenteritis confers an increased risk of IBS, which will be covered in the next section (2.2.3 Inflammation) [46]. This association leads to the hypothesis that FGID depends on a disturbed microbiota. The amount of research on microbiota and FGID has increased rapidly during the last decades, but most studies have been performed in adult populations. Some paediatric studies support that an altered gut microbiota is involved in paediatric IBS, but no single bacterial strain or genera has been identified as a single cause [47-49]. Bacterial diversity, that is a microflora composed of many different bacterial strains, appears to be desirable in many aspects [50].

The gastrointestinal tract from mouth to anus contains an immense number of bacteria and a vast immune system along with enteric innervation. These three systems; microbiota, the enteric immune system and the enteric nerve system exist alongside and have an intense interaction. The biochemical signalling between the gastrointestinal tract and the central nerve system is called the gut brain axis [51]. In the central nervous system, these processes supposedly interfere with pain experiences [52]. Still, the cause-effect between microbiota and FGID is not surely established, since it has not been studied prospectively in humans. Animal research supports that microbiota has an impact on the mind and behaviour [53, 54]. The mechanisms in the gut-brain-axis is supposedly caused partly by dysbiosis inducing an inflammatory response, thus interacting with the enteric nerve system [32, 33]. Studies on germfree animals have revealed that these animals show an exaggerated hypothalamic response to psychological stress, and that this increased response can be normalised with gut colonisation [55].

### 2.2.3 Inflammation

The strongest known risk factor for developing IBS is a previous episode of gastroenteritis. Associated factors that increase the risk are severity of initial illness, female sex and adverse psychological factors [46]. The magnitude of the increased risk for childhood IBS after gastroenteritis has been demonstrated to be twofold, or with a shorter follow-up time after the infection, as high as three to four-fold [56-58]. A childhood salmonella or giardia gastroenteritis in smaller studies has been associated to a three-fold increased risk of adult IBS, up to a decade after the episode [59-61]. Increased prevalence in functional dyspepsia (FD) after a viral gastroenteritis has been demonstrated in adult but not in paediatric studies [59, 62].

The postulated mechanism of low-grade inflammation in ap-FGID is seen in different signs of immune dysregulation. Differences have been found in small studies regarding gastrointestinal permeability, peripheral blood cytokines and inflammatory cell counts of intestinal biopsies in IBS (children) and in FD (adults), but these signs of inflammation are not macroscopically visible [33, 63-69]. Neither blood nor faecal samples from ap-FGID patients

display signs of general inflammation. Calprotectin, a calcium- and zinc-binding protein abundant in neutrophils, measured in faecal samples is widely used clinically in the diagnosis and management of inflammatory bowel diseases [70]. Childhood FGID was not related to elevated levels of calprotectin in a few studies, thus contradicting a significant inflammatory component in ap-FGID [71-73]. Calprotectin is a useful tool in discriminating FGID and IBD in children. A meta-analysis showed that adding calprotectin to the diagnostic workup of paediatric patients with chronic gastrointestinal symptoms improved the diagnostic efficacy of IBD [70].

### 2.2.4 Visceral hypersensitivity and motility

Physiological changes in the gastrointestinal tract usually do not induce pain. Visceral hyperalgesia in ap-FGID has been demonstrated in many controlled studies [74-76]. Children with ap-FGID had significantly lower thresholds than comparisons for both sensation and pain in response to rectal as well as gastric distension [76]. Motility disturbances presenting as delayed gastric emptying and abnormal antral motility have been found in children and linked to symptom severity of ap-FGIDs but also to the exposure to previous emotionally stressful events [77, 78]. Experimental data supports that early life pain or stress can induce impaired stress response and altered descending neuronal inhibitory control, with possible subsequent visceral hypersensitivity [79]. The role of serotonin in children with FGID is explored in intestinal biopsies which have revealed a higher serotonin content in IBS but not in FD, indicating that serotonin signalling plays a role in paediatric IBS pathogenesis [68, 80].

### 2.2.5 Lifestyle and coping style

Lifestyle characteristics such as diet and stress may induce epigenetic changes that may in turn be key factors in the development of chronic pain [81]. This can occur for example as DNA methylation or acetylation, without demonstrable changes in the genetic sequence [81]. The rise in incidence of obesity, cardiovascular disease and stress-related disorders are commonly regarded as associated to modern lifestyle and society's affluence [82]. The increased prevalence of allergies and other immune-related disorders are partly regarded due to an affluent society as well [83]. The hygiene hypothesis, although revised in the past years, regards a modern lifestyle with decreased enteric microbiota diversity responsible for this increase [84]. Paediatric IBD, also an immune-related disease, has increased during the last decade's [85]. In contrast, the prevalence of ap-FGID appears rather stable during the past half century.
Most studies suggest that RAP in children is associated to lower socioeconomic conditions [86-88]. In contrast, IBS in young adults was more common in those studying at University (medical students), as compared to matched controls not at university [86]. A large Japanese school survey found that consumption of alcohol, smoking, sleep disturbances and anxiety was more common in adolescents with IBS [89].
This thesis has explored he anthroposophic lifestyle in relation to the development of ap-FGID. We thought that it would be of interest to study the effect of this lifestyle, since diet, microbiota and exposure to stress are regarded as contributing etiologic agents in ap-FGID. The anthroposophic lifestyle is characterised by differences in diet, for example in consumption of lactic acid vegetables and more often a vegetarian, fibre-rich diet [90-92]. Furthermore, children of anthroposophic families have displayed differences in faecal microflora [91, 93, 94]. Finally, this lifestyle has also been related to lower cortisol levels in

infants, hypothetically due to lower exposure to external stressors during infancy [95]. These low cortisol levels predicted a lower risk of sensitisation [96]. The anthroposophic lifestyle is known to emphasize the importance of protecting infants from unpleasant stressors [97].

### 2.2.6 Food

Both eating and defecation may initiate abdominal pain. Therefore, food has been suggested to be the culprit for ap-FGID [98]. To study the role of food in the context of ap-FGID is difficult, but attempts have been made. Intake of “fast food” was associated to an increased prevalence of FGID in a cross-sectional study involving Chinese adolescents, presumably due to higher intake of trans-unsaturated fats [99]. Primary lactose intolerance depends on an inherited absence or reduced capacity of the small intestinal brush border enzyme lactase [100]. When lactose in milk is not digested by lactase into monosaccharides it acts as substrate for colonic bacteria. Common symptoms are abdominal pain, flatulence and watery, acid stools upon milk intake. Lactose intolerance is more common in some parts of the world but unusual in northern Europe and generally, symptoms are unusual before 5-6 years of age [100]. Vandenplas has stated that lactose intolerance decreases the quality of life, if not diet is reduced in lactose content, but it is not associated with "true disease" [101]. The symptoms abdominal pain and flatulence in lactose intolerance are considerably similar to those in diarrhoea-predominant IBS. A diet low in Fermentable Oligo-, Di- Mono-saccharides And Polyol, (FODMAP), reduces both the amount of lactose and wheat. It will be discussed more in detail in the Treatment section below. Gluten has become a publicly strong candidate in causing abdominal symptoms, and several double-blind placebo-controlled adult studies have been performed, with conflicting results [102-104]. Wheat, which contains gluten, is one out of many short-chain carbohydrates included in the FODMAP concept, but not the single cause of IBS. It is important not to confuse gluten sensitivity with celiac disease (CD), and to exclude CD before a disoder of non-celiac gluten sensitivity is considered. In children this must be emphasised, as CD is a life-long disorder with undebatable benefits of a strict diet. Adult patients with gluten sensitivity may decide about the diet for themselves depending on symptom intensity. The terms ”non-celiac wheat” or ”wheat protein” sensitivity or ”FODMAP sensitivity” has been proposed [105]. It is reasonable to believe that ap-FGID may be falsely regarded as an expression of lactose intolerance or wheat protein sensitivity. Such a false diagnosis may be especially frequent in children with diarrhoea-predominant IBS.

The food content may cause abdominal symptoms, but also eating habits can modify gastrointestinal sensation and motility [106]. A clear majority of adolescents with IBS have reported abdominal symptoms related to eating and that they stop, or restrict eating due to, or to prevent symptoms [107].

### 2.2.7 Psychological factors and stress

Psychological and social factors are important in childhood RAP and other health problems [108, 109]. Alfvén et. al. have defined psychosomatic abdominal pain according to the presence of predefined criteria. Examples of these criteria are: aggravation of chronic negative stress at the time of RAP onset, pain in parallel with chronic negative stress and feeling better or pain-

free during periods of no negative stress [110]. These stressful life events could account for the symptoms in nearly half of the children with RAP in their study[111]. Reduced, as well as higher cortisol levels have been shown in children with psychosomatic RAP, indicating a dysregulation of the HPA-axis [112, 113].

In a review on eight prognostic RAP studies, child factors like behavioural disturbances and depressive/anxiety disorders were explored in relation to prognosis [114]. They found no support for the hypothesis that these children factors predicted persistence of RAP. In the same review, there was moderate evidence that parental gastrointestinal problems were linked to an increased risk of persistence. A more recent prospective study revealed that individuals with RAP in childhood had a substantially increased risk of anxiety disorders in adolescence and young adulthood [115].

A key study linked to the expression "Disorders of the Gut Brain Interaction" was performed in Australia in a population-based setting on 1002 adults, published in 2012 [116]. FGID, IBS and FD were assessed on one hand and a validated inventory on depression and anxiety on the other. With a 12-year follow-up the study showed that FGID at baseline increased the risk of depression and anxiety 12 years later. Anxiety at baseline also entailed a small, but significantly increased risk of FGID 12 years later, and offers an elegant example that the brain-gut pathway in FGIDs is bidirectional [116]. An older but smaller study without control group, performed in 1996, reveals the possible role of the brain-gut-axis [117]. Adult in-patients treated for infectious gastroenteritis, thus a selected population at risk for developing post infectious IBS, were measured on psychometric scores at the initial illness. Six months later, patients who subsequently developed IBS symptoms, had scored higher on anxiety and depression at the time of acute infection, than those who did not develop IBS[117].

### 2.2.8 Exposure to adversities

Retrospective and cross-sectional studies have shown associations between negative life events, child abuse, sexual abuse, and abdominal pain [118-121]. One prospective study on childhood adversity showed no association to IBS at middle-age, but ap-FGID was not examined [122]. Another prospective study in a subpopulation of children at risk of maltreatment showed increased risk of FGID in children after sexual abuse [123]. Cross sectional and longitudinal studies have suggested that childhood RAP may be associated with parental anxiety and depression, most often assessed in mothers [8, 124-127]. In a recent study, parental pain-threat perceptions seemed to increase number of health care visits for their children with RAP [128].

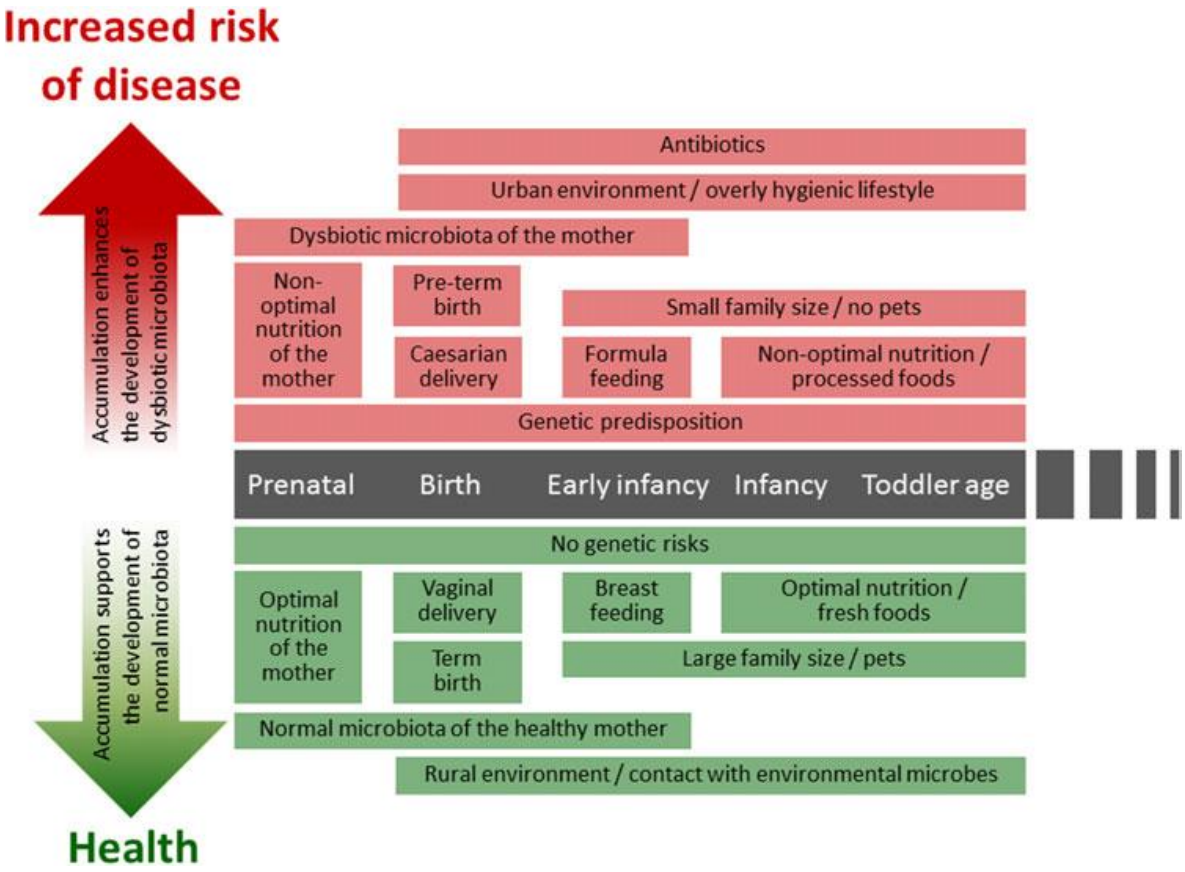


Figure 2. Proc Nutr Soc 2014;73:457-69. Downloaded from https://www.cambridge.org/core. Printed with permission.

## 2.3 PREVALENCE

### 2.3.1 Abdominal pain-related FGID

In Apley and Naish's classical study from 1958, the prevalence of RAP was 11 % [11]. In the most recent review on functional abdominal pain disorders in children, the prevalence ranged from 2-31 %, as defined by Apley, Rome II or Rome III [129]. The pooled prevalence was 13.5 %. In a review on 26 FGID studies by Boronat et.al., IBS with a prevalence of 9 %, seemed to be the most common presentation of the ap-FGIDs [130]. Prevalence has been investigated all over the world, with most studies performed in North America and Asia. In recent years, studies from Africa (Nigeria) have been published [131, 132]. Boronat et. al. (2017) found no evident difference concerning overall prevalence rates over the world [130]. Korterink et. al. (2015), on the other side, reported higher pooled prevalence rates in South America (16.8 %) and Asia (16.5 %) compared to Europe (10.5 %) [129, 130]. A large school-based study was performed in Japan, but only IBS was assessed with an unexpected high prevalence, 19 %, but also a great increase in prevalence rates was seen between 12 and 17 years, from 10 % to 26 % [89].

Prevalence differences between girls and boys were seen already in Apley's study. In early childhood there were similar prevalence rates but from the age of 8 years RAP was increasingly common in girls. Later studies also display higher female prevalence rates. Girls also more frequently report multiple pain locations, like back-pain, headache and generalised pain [133].

It is difficult to compare the prevalence of RAP both over time and between countries. One reason is that definitions have changed during the years, from Apley to Rome IV. Nevertheless, percentages are rather stable, not indicating any major fluctuations in the prevalence of ap-FGID. With an increase in prematurity and accompanying invasive perinatal actions, a rise in the prevalence during childhood could be expected but has not yet appeared. The choice of method for measuring, through self-reported questionnaires or parental questionnaires, yields different numbers. Parental report most likely underestimates prevalence of RAP in adolescents [134].

Most children with gastrointestinal complaints are diagnosed and treated in primary care [135]. In studies from Australia and the Netherlands 2-5 % of all paediatric visits in primary care were related to abdominal pain [4, 135]. In unpublished Swedish data the proportion was similar [136]. In 2012, abdominal pain was the second most common cause for seeking the paediatric emergency department at the Karolinska University Hospital in Stockholm (Boston Consulting Group, Utredning av framtidens hälso- och sjukvård [Investigation on future healthcare] 2012).

### 2.3.2 Overall FGID

In student samples, FGID prevalence ranges from 10-29 %, when defined according to Rome II, III or IV criteria [130]. The first survey based on Rome IV criteria showed that one out of four infants, toddlers, children and adolescents fulfilled symptom-based criteria for a FGID in America [40]. The study was performed as an online interview with mothers and not on self-reported symptoms from adolescents.

## 2.4 TREATMENT

Developing new tools for treatment of children with ap-FGID is a delicate task. It is often a chronic disorder but fluctuating and eventually with long asymptomatic periods. This means that the probability that a child with ap-FGID will improve on any treatment is substantial. Therefore, it is crucial that research should be performed in randomised, controlled treatment-studies. Another important issue is the placebo effect. A review on childhood ap-FGID found that approximately four out of ten children improve on placebo [137]. Furthermore, due to the basically benign nature of RAP, treatment should not introduce risks or adverse events.

The effect of treatment can be measured as pain reduction but also as stooling, number of health-care visits, degree of school absence and quality of life. Quality of life has been shown to be reduced in children with ap-FGID in many studies [40, 138, 139]. It seems reasonable to believe that high-quality health care, not necessarily medical investigations, spent on this group of patients would create major improvements for both childhood health and health economy [6, 7, 9]. The physician-patient interaction in IBS is most probably important [140]. A careful and accurate consultation in children with RAP is also crucial for discovering any kind of abuse or maltreatment.

A few studies have attempted to identify the children with FGIDs who are at greatest risk for persistent pain-related impairment [141-143]. A major finding was that if both parent and child reported presence of clinically significant child anxiety, as compared to discordant reports or low anxiety, this was related to an increased future impairment [142]. Children without relationship between pain and stooling were more prone to a future decreased pain frequency [143]. In the future it may be possible to focus treatment on children at highest risk of developing chronic pain and disability.

### 2.4.1 Pharmacology

There is no distinctly evidence based pharmacological treatment in ap-FGID [144, 145]. Rather often it is difficult to decide if a child with recurrent abdominal pain suffers from constipation or IBS. Therefore, the initial treatment of a child with RAP is often laxatives. If pain disappears when the child has received accurate laxative treatment, the diagnosis is constipation. If pain is diminished but not absent, the diagnosis might be IBS with constipation.

Even if prescribed fibre supplements and bulking agents are not evidence-based for treatments of pain in ap-FGID, they seem to be of help for some patients. At least the alliance between doctor and patient is improved [146]. There is no analgesic treatment that has been proved effective in ap-FGID. Antidepressant treatment is often used in adult IBS [147]. Treatment with antidepressants to patients 6-18 years with IBS has been tried in a few randomised trials. The placebo effect was more than 50 %, comparable to treatment with amitriptyline [148]. In a few small studies, improved quality of life was found in relation to antidepressant treatment to IBS, but the treatment had no effects on gastrointestinal symptoms. Therefore, antidepressant treatment to young people with IBS should only be used in selected cases or in controlled studies.

Peppermint oil is a calcium-blocker available in herbal compounds and studied in only one randomised controlled study in children [149]. The severity of gastrointestinal symptoms was significantly more reduced in the peppermint oil treatment group than in placebo. Proton pump inhibitors are frequently prescribed to children with ap-FGID [150], but only one paediatric study found that another gastric pH-modifying compound, famotidine was effective against both reflux disease and functional dyspepsia [151].

### 2.4.2 Psychology

The management of children with ap-FGID often requires an interdisciplinary approach, especially in cases with great suffering and frequent school absence [9]. Contact with a psychologist is sometimes recommended after the diagnosis has been set. Some families decline psychiatric or psychological contact and yet others accept but the psychiatric care does not always have an effective treatment. Worldwide there are promising psychological treatment methods that are investigated scientifically [146, 152, 153].

#### 2.4.2.1 Cognitive behavioural therapy

Cognitive behavioural treatment (CBT) in ap-FGID can have many different components, for example relaxation, cognitive techniques for changing thoughts or distraction [145]. CBT can also use methods with exposure to gastrointestinal symptoms through exercise involving reduced avoidance of activities that induce symptoms [154]. CBT in adults with IBS has demonstrated considerable efficacy in several studies and is broadly accepted in clinical use [155]. Internet-delivered, exposure-based CBT against ap-FGID has been studied in children 8-17 years old, with promising results, measured as total score on Gastrointestinal Symptoms Rating Scale for IBS [156, 157]. A three-session telephone intervention for cognitive behavioural therapy with parents proved to be as effective as the same intervention face to face for children 7-12 years. Outcomes were parental solicitousness, pain beliefs, catastrophizing, child-reported coping and functional disability [158].

#### 2.4.2.2 Hypnotherapy

Treatment for ap-FGID with hypnotherapy has been subject to several randomised, controlled studies in children [159-162]. Hypnotherapy to patients with ap-FGID is performed by a trained therapist. It includes general relaxation, abdominal pain control and ego-strengthening suggestions [161]. A protocol adapted for children has been used, with six sessions of 50 minutes each, during a three-month period. The sessions can be combined with home exercises sustained by an audio compact disc. Audio compact disc has also been used separately for home treatment [160]. Hypnosis by a therapist is expensive and time-consuming, and therefore home-hypnosis with the assistance of a compact-disc-player could increase accessibility [159].

Vlieger et. al. performed a randomised controlled study comparing hypnotherapy with standard medical treatment in children with FAP or IBS aged 8-18 years. They found a reduction in abdominal pain frequency and pain intensity scores, that was significantly greater in the hypnotherapy group. At one-year follow-up, successful treatment was accomplished in 85 % of the hypnotherapy group and 25 % of the standard medical treatment group. Other studies on hypnotherapy to children with FAP and IBS have displayed

improvements in quality of life and reductions in school absenteeism [152]. The beneficial effects of hypnotherapy have been sustained up to five years after treatment [162].

### 2.4.3 Diet

As mentioned earlier, food is often experienced as the culprit in ap-FGID. Dietary treatment of children with ap-FGID should always be performed in cooperation with specially educated, paediatric dieticians, with knowledge of the nutritional needs of growing children and adolescents. Since many children and adolescents with IBS associate abdominal pain with the intake of certain food products, voluntary avoidance is common. Dairy and grains are the most commonly avoided foods in children with IBS [163]. A limited period of milk protein or lactose elimination, in cooperation with a doctor or dietician, may be indicated, since the only reliable diagnosis in allergy and food intolerance is elimination and provocation.

The food content can cause intestinal, luminal distension and thereby bloating and pain in those with visceral hypersensitivity and abnormal gut motility, which has been shown in IBS. Dietary treatments often aim to minimize bacterial fermentation through providing less substrate. There are two Cochrane reviews on diet interventions for ap-FGID in children from 2008 and 2017 respectively [164, 165]. In 2008 there were no high-quality studies on the topic. Newlove-Delgado et. al. state in 2017 that the evidence for probiotic therapy in IBS is moderate, and that there is insufficient evidence for both fibres and FODMAP [164]. A low FODMAP diet reduces pain in adult IBS but is not enough studied in children [146, 166, 167]. It has been proposed that the response to diet treatment is dependent on host microbiota, more specific the saccharolytic capacity [168]. A clinical observation is that children who seek care for abdominal pain often associates the pain with certain foods. An interesting study on food avoidance found that it was more common in adolescents with IBS than in their healthy peers to identify at least one self-perceived food intolerance (92.9 % versus 62.5 %). The number of self-perceived food intolerances was weakly associated to severity of IBS [163].

The first randomised, controlled study on vitamin D supplementation to adolescents with IBS was published this year. Subjects were 14-18 years old with IBS according to Rome III and a vitamin D level of mean 17.2 ng/ml [169]. Vitamin D supplementation improved both symptoms and quality of life in subjects with IBS and Vitamin D deficiency, but mechanisms are unclear, and results need to be confirmed.

### 2.4.4 Probiotics

A specific dietary treatment is supplementation with probiotics. Probiotics do not require a doctor's prescription and can be administered as pills, capsules, liquids and powders. Foods enriched with probiotic bacteria is common in infant formula, yoghurt, cereals etc. [170]. Probiotics is a concept that has emerged in the last decades and the definition is varying. The WHO definition is "*live microorganisms which, when administered in adequate amounts, confer a health benefit on the host"*. Regulations on the number of colony forming units that must be included to be called probiotic varies between different countries. It is generally

believed that $10^8$ -$10^{10}$ viable bacteria/day should be administered to be clinically effective [170]. Common probiotic strains are *lactobacillus, bifidobacterium* and *streptococcus*.

The treatment effects of probiotics are thought to depend on the effects on microbiota, which has been described in 2.2.2. Experiments in animals and humans have been able to show that the microbiota is altered upon administration of probiotics [52, 171]. The mechanism of probiotics on gastrointestinal function is conveyed through interference with pathogens, improvement of intestinal barrier, immunomodulation and on neurotransmitter production [52, 171]. The treatment of rotavirus-enteritis in children is an example on how probiotic treatment can confer health benefits, although the magnitude of effect is moderate [172].

Treatment with probiotics to children and adolescents with ap-FGID has shown promising results. It should be noted that this treatment seems to be free of adverse effects. Randomised, controlled studies to children between 4 and 18 years have shown significant improvement in frequency and duration of pain as compared to placebo treatment [173, 174]. The most consistent results have been observed in relation to treatment of IBS and not of FAP or FD. Limitations are that the treatment groups have received different probiotic strains and therefore pooling of data and meta-analysis are difficult to perform [175]. It also worth noting that the required duration of treatment is unknown. In clinical trials 4-8 weeks treatment duration is usual [173, 174].

Prebiotics are non-digestible carbohydrate fibres, but not as well defined as probiotics. Prebiotics are simply food for the probiotic bacteria and interesting as new treatments for ap-FGID, but not enough studied [176]. Food fibres can be regarded as prebiotic. In the Cochrane report on dietary treatment of paediatric IBS, fibre interventions with psyllium, guar gum and glucomannan, were compared with placebo. They seemed to improve children with IBS, but the quality of these studies was deemed low [164].

# 3 AIMS

The overall aim of this thesis was to evaluate a diagnostic questionnaire, to explore risk factors and to study prognosis in abdominal pain-related functional gastrointestinal disorders in childhood. Specifically, we aimed:

- To evaluate the sensitivity, specificity, and positive predictive value of a Rome III-based questionnaire in diagnosing abdominal pain-related functional gastrointestinal disorders (ap-FGID) when administered to children seeking care for gastrointestinal symptoms (Study I)
- To explore how alarm symptoms used in different combinations together with the Rome III questionnaire affect the sensitivity, specificity, and positive predictive value in diagnosing ap-FGID (Study I)
- To study if overall antibiotic treatment, types of antibiotic substances used, and number of antibiotic courses during childhood affects the risk of ap-FGID at 12 years of age (Study II)
- To test the hypothesis that family lifestyle is associated with the development of ap-FGID at five years of age and to evaluate if any specific lifestyle characteristic could explain any such association (Study III)
- To study if RAP in early childhood or at 12 years of age are risk factors for later paediatric RAP or ap-FGID (Study IV)
- To examine prevalence and turnover of childhood RAP between one and 16 years of age (Study IV)

# 4 SUBJECTS AND METHODS

Paper I was based on a prospective questionnaire validation study performed at paediatric outpatient centres and university hospitals. Papers II and IV were performed in the birth cohort BAMSE and paper III in the birth cohort ALADDIN.

## 4.1 STUDY I. ROME III QUESTIONNAIRE STUDY

### 4.1.1 Subjects, inclusion criteria

We invited 310 children aged 4-17 years to take part in the study. Invited children were attending paediatric outpatient centres in Stockholm between January 2013 and May 2014, altogether seven centres.

- A paediatric consultation because of gastrointestinal complaints
- Signed consent from both parents
- 83 % of those initially eligible were included in the main analysis, n=258

### 4.1.2 Methods

#### 4.1.2.1 Questionnaires

The sources of data consisted of information from questionnaires at inclusion, data from patient records and a diagnostic review.

- Questions (yes/no) concerning eight gastrointestinal alarm symptoms during the two months preceding the visit (Table 4)
- A validated Swedish version of the Questionnaire on Paediatric Gastrointestinal Symptoms Rome III
- Since three of the alarm symptoms (heredity for organic gastroinestinal disease, defecation at night and bloody stools) were associated with organic gastrointestinal disease (OGID) in our study, a missing answer on these items excluded the child from further analysis. The other five alarm symptoms did not show any significant association with OGID and a missing answer on these items was therefore regarded as negative

#### 4.1.2.2 Patient records

Data extraction 6-12 months after inclusion:

- Number of previous visits for gastrointestinal complaints
- Specified laboratory tests
- Diagnostic radiology
- Endoscopies and bowel biopsy analyses
- Clinical diagnoses and prescribed treatments
- Median patient time covered 2.7 years

| Table 4. ALARM SYMPTOMS |
| --- |
| Heredity for gastrointestinal disease |
| Defecation at night |
| Bloody stools |
| Waking up at night due to abdominal pain |
| Right-sided abdominal pain |
| Unintentional weight loss |
| Painful swallowing |
| Unexplained fever |

#### 4.1.2.3 Diagnostic review

The most likely clinical diagnosis was set through revision of patient records by two experienced paediatric gastroenterologists performed at least 12 months after the study inclusion. They were blinded to the answers to the questionnaires. In 249 of 258 cases (97 %) there was initial agreement between the two reviewers on whether the cause of gastrointestinal complaints had an organic or functional origin. The nine patient records where there was initial discordance, were re-reviewed until a common diagnosis was agreed upon in all these patients.

This clinical diagnosis was regarded as the reference when assessing the ability for Rome III symptom profiles, alarm symptoms and laboratory tests to discriminate between functional and organic causes of gastrointestinal complaints.

#### 4.1.2.4 Statistics

The capacity of the Rome III symptom criteria to identify abdominal pain-related functional gastrointestinal disorders (ap-FGID), alone or in combination with negative alarm symptoms or normal laboratory tests, was tested. The reference was the diagnosis set through a record review one year after administration of the questionnaire. Sensitivity, specificity, positive and negative predictive values were calculated in four field tables.

The questionnaire answers provided information to classify whether children fulfilled the Rome III criteria for ap-FGID, but not for other functional gastrointestinal disorders (FGID), for example constipation, vomiting and nausea. Accordingly, the children with a reference FGID other than ap-FGID (n=80) were excluded from the main analysis.

The remaining patients were categorised according to three different variables: positive paediatric Rome III symptom criteria, negative alarm symptoms and normal laboratory tests regarding faecal calprotectin (<100 µg/g) and serum Immunoglobulin-A-transglutaminase antibodies (IgA-TGA ab).

Four different combinations were subsequently compared with the reference diagnosis:

1) Subjects meeting the positive paediatric Rome III symptom criteria
2) Subjects meeting criteria in 1) plus negative alarm symptoms
3) Subjects meeting criteria in 1) plus fecal calprotectin <100 µg/g and normal IgA-TGA ab
4) Subjects meeting criteria in 1) plus both negative alarm symptoms and fecal calprotectin <100 µg/g and normal IgA-TGA ab

## 4.2 STUDY II. BAMSE COHORT

The BAMSE-project (Swedish acronym for children [Barn], Allergy, Milieu, Stockholm, Epidemiology) invited the parents of 7221 new-born children living in urban and suburban Stockholm between 1994-1996 to participate in a prospective study [177]. The aims of the BAMSE project were to establish risk factors for asthma and other allergic diseases in childhood, and to study factors of importance for prognosis at already established allergic disease. The original cohort comprised of 4089 children, whose parents fulfilled the two-month-old baseline questionnaire. The follow up rates for parental and child participation were 96 % (3925/4089) and 94 % (3843/4089) at 12 and at 24 months respectively. The follow up rate for adolescent participation at 12 and 16 years of age were 68 % (2795/4089) and 76 % (3115//4089) respectively of the original BAMSE cohort. At 12 and 16 years of age it was possible to answer web-based questionnaires. At 12 and 16 years of age adolescents and parents answered separate questionnaires.

### 4.2.1 Subjects and inclusion criteria

- The child had answered the question about gastrointestinal symptoms at 12 years
- No parental report of inflammatory bowel disease (IBD) and/or celiac disease (CD) in parental questionnaire at 12 years of age
- Number of children fulfilling the inclusion criteria, n=2732
- Flowchart in Figure 3

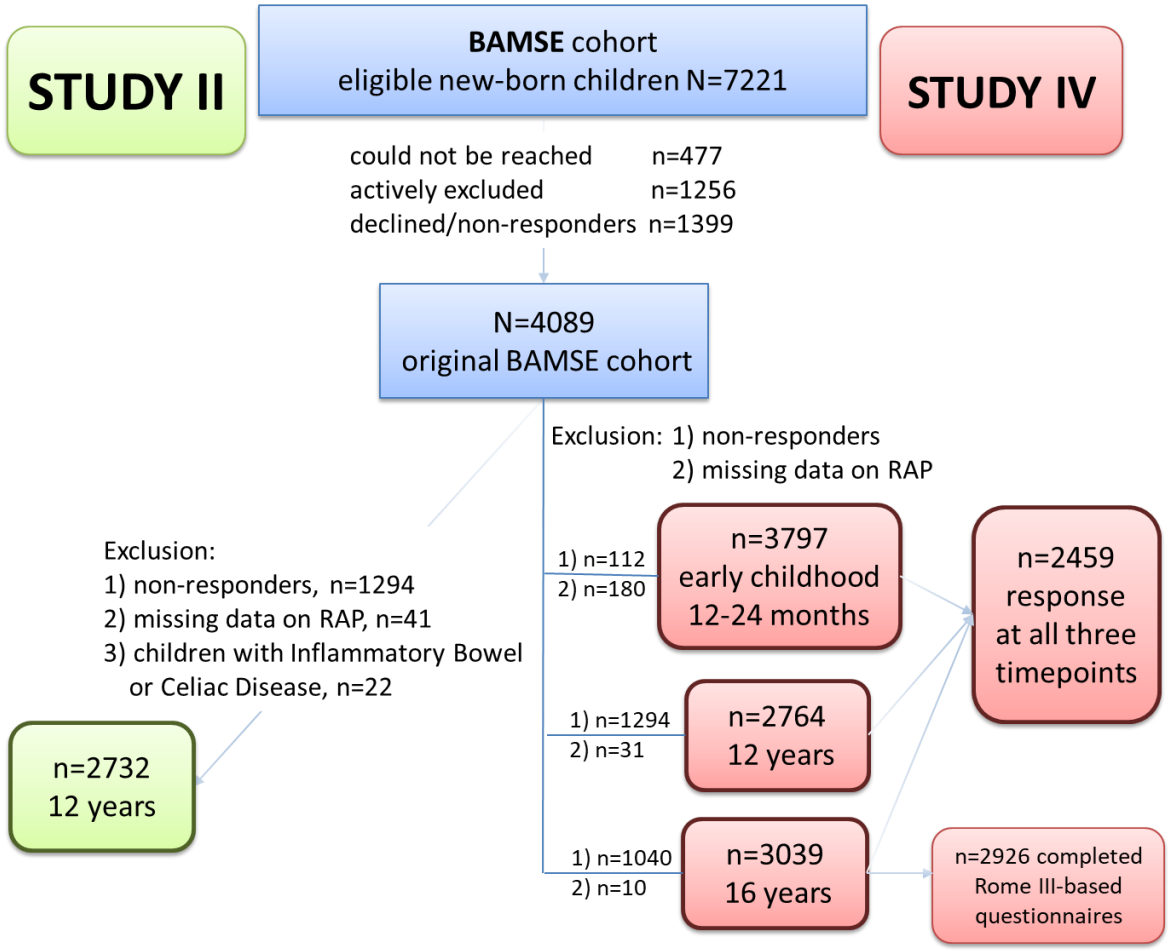


Figure 3. Flowchart BAMSE cohort

### 4.2.2 Methods

#### 4.2.2.1 Antibiotic exposure, questionnaire data

Information about antibiotic exposure in early childhood was collected from parental questionnaires at child ages of 12 and 24 months [178]. Children with no parent-reported antibiotic use in the first two years of life were considered reference group. Children who had received antibiotics were divided into three groups according to antibiotic exposure during:

- The first but not the second year of life
- The second but not the first year of life
- Both the first and the second year of life

#### 4.2.2.2 Antibiotic exposures, The Swedish Prescribed Drug Register

Information about dispensed antibiotics from 9 to 12 years of age was collected through record linkage to the Swedish Prescribed Drug Register by using the Swedish personal identity number [179]. Antibiotics administered in hospital are not recorded in the register.

Use of antibiotics in the three years preceding the outcome RAP at 12 years of age was classified in four different ways:

- Any antibiotic use in the three years preceding the outcome
- Number of antibiotic courses: one course, two courses or ≥3 courses
- Timing of last antibiotic course: up to five months ago, six to 11 months ago or 12 to 36 months ago
- Type of antibiotic substance regarding the six most frequently dispensed antibiotics: phenoxymetylpenicillin, flukloxacillin, cefadroxil, amoxicillin, erythromycin or tetracycline

#### 4.2.2.3 Definition of RAP

- Children with monthly abdominal pain and no parent-report of doctor's diagnosis of IBD and/or CD before 12 years
- Post-hoc analyses were performed for weekly abdominal pain with no parent-report of doctor's diagnosis of IBD and/or CD before 12 years

#### 4.2.2.4 Statistics and Confounders

Multivariable logistic regression analysis was used to assess the risk of RAP at 12 years.

Confounders were

- Asthma at one year, defined as at least three episodes of wheeze after three months and up to one year of age in combination with treatment with inhaled glucocorticoids and/or signs of suspected hyperactivity without concurrent upper respiratory infection
- Asthma at 12 years, defined as at least four episodes of wheeze in the last 12 months or at least one episode of wheeze during the same period, combined with prescription of inhaled glucocorticoids for symptoms of asthma
- Sex

### 4.3 STUDY III. ALADDIN COHORT

The ALADDIN birth cohort study (Assessment of Lifestyle and Allergic Disease During Infancy) has recruited families at anthroposophic and conventional maternal-child health centres [92]. The children were followed prospectively from infancy to five years of age, with parental questionnaires, clinical visits and biological samplings at regular intervals. Focus in the ALADDIN study is to assess development of allergy related disease in children of different lifestyles: anthroposophic, partly anthroposophic and non-anthroposophic. In study III, children from ALADDINs first recruitment wave (330 families) were supplemented with

140 children from a second wave. This subgroup of 470 children from the cohort were recruited in 2004-2009 (Figure 4).

### 4.3.1 Subjects and inclusion criteria

- The parents had completed questionnaire data regarding all items on lifestyle
- The parents had completed questionnaire data on abdominal pain
- From the 354 children thus identified, the following children were excluded:
    - Children with constipation according to Rome III criteria (n=31)
    - Children with infrequent abdominal pain, less than once a week, or abdominal pain with a duration shorter than two months (n=131)
    - Child with cow's milk protein allergy with gastrointestinal symptoms (n=1)
- The remaining study population thus comprised 191 children, who were further categorised into three lifestyle groups: non-anthroposophic (n=66) partly anthroposophic (n=75) and anthroposophic (n=50)

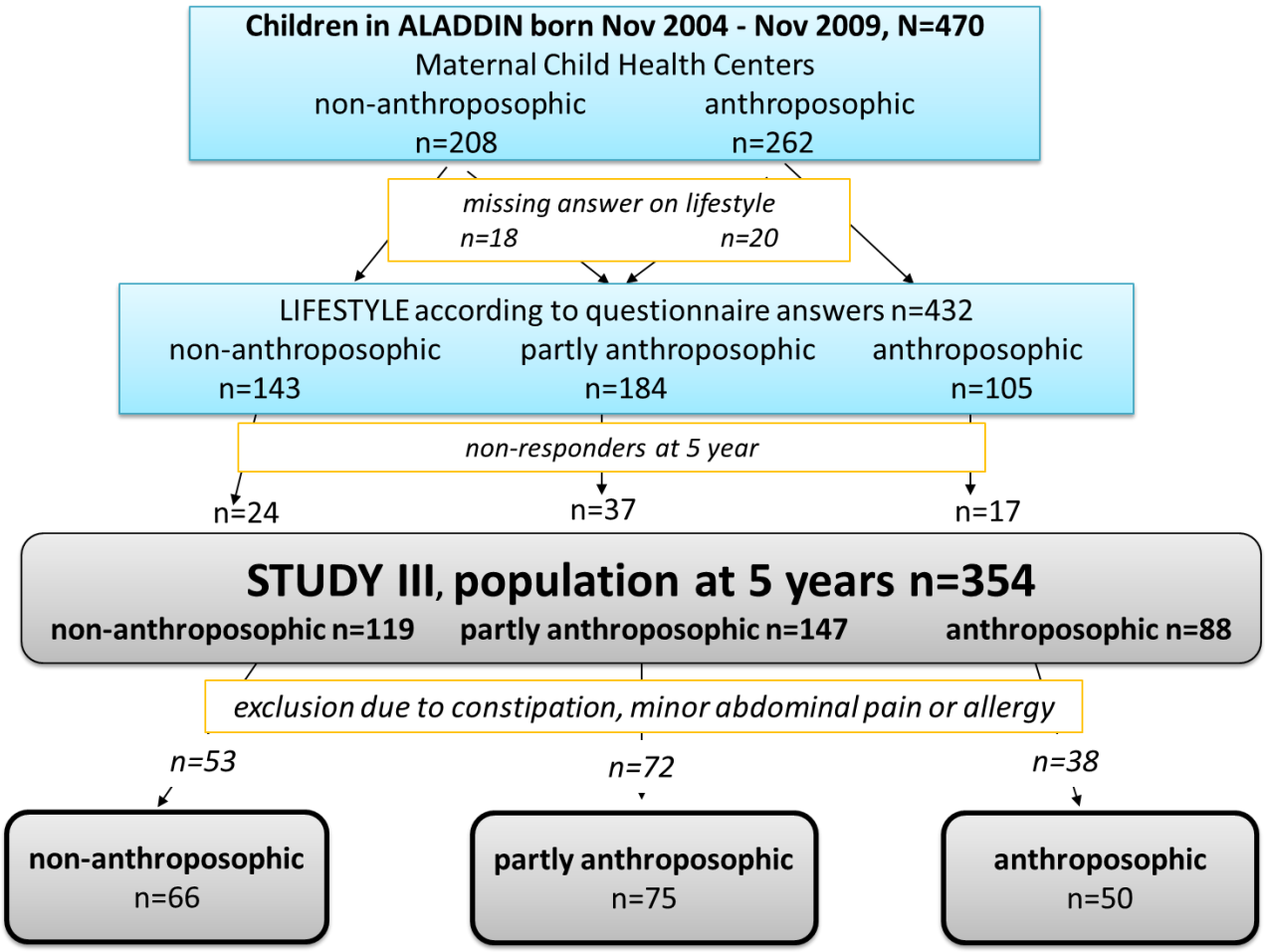


Figure 4 Flowchart ALADDIN cohort

### 4.3.2 Methods

#### 4.3.2.1 Questionnaires

- Lifestyle categorisations were based on parental responses to four questions:
    1. Had chosen an anthroposophic Maternal and Child health Centre
    2. Had planned anthroposophic child care
    3. At least one parent adhered to an anthroposophic lifestyle
    4. Everyday family life was influenced by an anthroposophic view of life

    Responding yes to all four items was categorised as anthroposophic and no to all items as non-anthroposophic. All other alternatives were categorised as partly anthroposophic.

- At child's age of five years, the parents filled in an adapted Swedish version of the Questionnaire on Paediatric Gastrointestinal Symptoms Rome III (QPGS-RIII)

#### 4.3.2.2 Definitions of Irritable bowel syndrome (IBS), Functional dyspepsia (FD) and Functional abdominal pain (FAP)

In all children whose parents stated that the child had abdominal pain at least once every week since at least two months a structured telephone interview was performed with one of the parents. The following issues were considered:

- Whether the questionnaire answers could be verified
- Occurrence of alarm symptoms
- Previous healthcare contacts

The main outcome in study III was abdominal pain defined according to the paediatric Rome III criteria of IBS, FAP and FD. FD and FAP were analysed together since the only difference between them according to Rome III is pain localisation and for children five years and younger it is difficult to localise abdominal pain.

#### 4.3.2.3 Lifestyle characteristics

- Birth (Caesarean section, home delivery)
- Family characteristics (older siblings, parents born outside Europe, parents with University degree and age at child's birth)
- Child at two months of age (parents smoke, formula during first week, breastfeeding for less than two months, colic, vitamin D supplements, any antibiotic treatment)
- Child at six months of age (breastfeeding for less than six months, colic, vitamin D supplements)
- Child at 24 months of age (fermented food, vegetarian food, in preschool)
- Child at five years of age (fermented food, vegetarian food, parent's divorce, in preschool, any antibiotic treatment and number of antibiotic treatments)

## 4.4 STUDY IV. BAMSE COHORT

The BAMSE-cohort was the basis for study IV (Flowchart Figure 3).

### 4.4.1 Subjects and inclusion criteria

- Parental participation at baseline, child's age two months
- Parental participation at 12 or at 24 months of age (at early childhood follow-up)
- Child participation at 12 years of age (at 12-year follow-up)
- Child and parental participation at 16 years of age (at 16-year follow-up)
- Exclusion criterion at early childhood was missing data regarding RAP at either 12 or 24 months, if combined with no RAP at the other early childhood assessment point

Multiple assessment points with different follow-up rates were defined:

- Early childhood, pooled data from questionnaires at 12 and 24 months, 93 % (n=3797)
- At 12 years of age, 68 % (n=2764)
- At 16 years of age, 74 % (n=3039)
- Complete follow up at all three assessment points, 60 % (n=2459)

### 4.4.2 Methods

#### 4.4.2.1 Questionnaires

- At 12 and 24 months, parents were asked if their child had "repeated attacks of colic" during the previous six and 12 months respectively
- At 12 years, children were asked if they had "recurrent abdominal pain, menstrual cramps excluded" and how often it occurred
- At 16 years, children were asked "How often – in the past two months – have you felt pain or discomfort in your stomach?"
- At 16 years, questions based on the ROME III Questionnaire on Pediatric Gastrointestinal Symptoms were included

#### 4.4.2.2 Definitions of RAP, IBS, FD and FAP

- If parents responded yes that their child had "repeated attacks of colic" either at the 12 month-questionnaire or at the 24 month-questionnaire, or both, the child was classified as having RAP in early childhood
- Children who had at least *weekly* abdominal pain with no parent-report of doctor's diagnosis of IBD and/or CD until the time of the questionnaire, were classified as having RAP at 12 years
- Children with at least *weekly* abdominal pain or discomfort, and no parent-report of doctor's diagnosis of IBD and/or CD in the questionnaires at 12-years and 16-years, were classified as having RAP at 16 years

- The questionnaire at 16 years allowed us to categorise children affected with ap-FGID at 16 years into irritable bowel syndrome (IBS), functional dyspepsia (FD) and functional abdominal pain (FAP), all according to the ROME III criteria (Table 2, page 10 and Table 3, page 12)

#### 4.4.2.3 Statistics

- Cross-sectional prevalence estimates were calculated by dividing children with a report of RAP or ap-FGID with the total number of children with that information available at each respective assessment point
- The period prevalence was calculated as the proportion of children with at least one report of RAP in any of the three assessment points divided by the total number of children with any report of RAP or with a consistent negative report at all three assessment points, 66 % of original cohort (n=2698)
- Turnover of RAP was analysed in children with complete follow up at early childhood, 12 and 16 years by describing the number of incident cases (no prior report of RAP), persistent cases (RAP at two consecutive assessment points) and cases in remission (RAP at the previous, but not current assessment point) at each assessment point
- For longitudinal risk estimates we used all observations available

#### 4.4.2.4 Confounders

- Asthma at one year, defined as in Study II
- Asthma at 12 years was defined as as in Study II
- Sex

# 5 RESULTS

## 5.1 STUDY I

### 5.1.1 Questionnaire-based Rome III criteria diagnoses

The questionnaire-based symptom profile of a functional gastrointestinal disorders (FGID) according to Rome III criteria was filled in for 72 % of all study children. One or more alarm symptoms were present in 81 % of the participants. Three of the alarm symptoms were significantly more common in children with organic gastrointestinal disease (OGID). These were heredity for IBD, CD or peptic ulcer disease ($p=0.017$), defecation at night ($p=0.028$) and blood in stools ($p<0.001$).

### 5.1.2 Patient records

The mean number of previous visits for abdominal complaints was 1.9 (range 0-5 visits). Laboratory testing was performed at least once in most children, including IgA-TGA ab (93 %), blood counts (92 %) and faecal calprotectin (59 %). Each one of these tests was significantly more likely to be normal in children with FGID compared to those with OGID ($p<0.02$).

### 5.1.3 Diagnostic review

FGID was diagnosed in 84 % (216/258). More than half, 54 % of the participants had abdominal pain-related FGID (ap-FGID) and in this subgroup of 140 children, 57 had irritable bowel syndrome, 59 had functional abdominal pain and 24 were diagnosed with functional dyspepsia. OGID was diagnosed in 42 of 258 children (16 %). Inflammatory bowel disease (IBD) was diagnosed before entering the study in two children and another five were diagnosed after inclusion. Celiac disease (CD) was diagnosed in 18 children. Concomitant FGID and OGID was present in nine children, all with celiac disease, and thus excluded from all the analyses on FGID.

### 5.1.4 Evaluation of Rome III symptom criteria

The ability of a Rome III-based questionnaire to accurately diagnose an ap-FGID was calculated in a four-field table. When using plain positive Rome III symptom criteria for ap-FGID, the sensitivity was 0.78 and the specificity 0.32. When positive Rome III criteria for ap-FGID were combined with the absence of alarm symptoms, the sensitivity was 0.15 and specificity 0.90 (Table 5). When positive Rome III criteria for ap-FGID were combined with negative IgA-TGA ab and faecal calprotectin <100 µg/g, we found a sensitivity of 0.56 and a specificity of 0.85. When positive Rome III criteria for ap-FGID were combined with absent alarm symptoms, negative IgA-TGA ab and faecal calprotectin <100 µg/g, we found a sensitivity of 0.15 and a specificity of 0.89. We also combined positive Rome III criteria for ap-FGID with the absence of only the three alarm symptoms that were significantly associated with OGID - heredity, defecation at night and blood in stools. The sensitivity was then 0.35 and specificity 0.86. When we included subjects with non-pain-related FGID, namely constipation, diarrhoea and vomiting, in the analysis, the sensitivity for plain positive Rome III criteria for ap-FGID was 0.78 and the specificity was 0.34.

**Table 5. Functional cause of abdominal pain according to questionnaire (Rome III criteria) combined with negative alarm symptoms (yes/no) in relation to clinical diagnosis after record review (ap-FGID or Organic disease)**

| Rome III and negative alarm symptoms | Clinical ap-FGID | Clinical organic disease | Total |
|---|---|---|---|
| **Yes** | 20 | 4 | 24 |
| **No** | 112 | 36 | 148 |
| **Total** | 132 | 40 | 172 |
| **Sensitivity: 20/132=0.15**<br>**Specificity: 36/40=0.90**<br>**Positive predictive value: 20/24=0.83**<br>**Negative predictive value: 36/148=0.24** | | | |

## 5.2 STUDY II

### 5.2.1 Prevalence of antibiotic use and RAP

According to questionnaire data, 72 % of the children received antibiotic treatment in the first two years of life. Antibiotic treatment during the first but not the second year of life occurred in 10 % of the children, during the second but not the first year of life in 29 % and during both the first and the second year of life in 32 %. In the three years preceding the 12 years of age questionnaire, 38 % of the study participants were dispensed a course of a prescribed antibiotic drug according to the Swedish Prescribed Drug Register. The mean number of dispensed antibiotic courses to all study children during the three-year period was 0.7 (range 0-83 courses). Of the 1045 children who were dispensed a prescribed antibiotic course in the three-year period, 198 (7.3 %) were dispensed three or more courses. The most common antibiotic courses dispensed were substances commonly used for treatment of airway related diseases such as otitis, tonsillitis, bronchitis and pneumonia. Only 1.5 % of the children were dispensed a prescribed broad-spectrum antibiotic from the tetracycline group.

The prevalence of monthly RAP at 12 years was 9 % and of weekly RAP 4 %.

### 5.2.2 Antibiotic use and risk of RAP

#### 5.2.2.1 In early childhood:

Antibiotic treatment in the first two years of life did not affect the risk of monthly RAP at 12 years of age in analyses of the whole study group. Stratified analyses showed that girls, but

not boys, who received antibiotics during both the first and the second year of life, had an increased risk of RAP at 12 years (OR 1.65; 95 % CI: 1.09-2.49). In all other stratified analyses, no significant associations were found.

#### 5.2.2.2 Between 9 and 12 years:

One, two or three antibiotic courses in the three preceding years was not associated with an increased risk of monthly RAP at 12 years. In post-hoc analyses three or more antibiotic courses in the three preceding years showed an increased risk of weekly RAP (crude OR 1.97; 95 % CI: 1.12-3.46).

There was no significant association between any of the examined types of antibiotic substances and RAP. However, use of tetracycline was associated with a non-significant increased risk of monthly RAP at 12 years of age (OR 1.80; 95 % CI: 0.74-4.34).

The time between the last dispensed antibiotic course and the outcome questionnaire at 12 years of age did not affect the risk of RAP.

Antibiotic use in all the three time periods (0-1 year, 1-2 years and 9-12 years) and RAP at 12 years of age showed no association (OR 1.03; 95 % CI: 0.70-1.54).

## 5.3 STUDY III

### 5.3.1 Prevalence of ap-FGID

The prevalence of ap-FGID at five years was 15 % (54/354) in the total ALADDIN cohort. IBS was present in In most cases the duration was more than one year. Of the 191 children who were selected for analyses in relation to their lifestyle, 17 % had ap-FGID in the non-anthroposophic lifestyle study group, 33 % in the partly anthroposophic group and 36 % in the anthroposophic group.

### 5.3.2 Risk of ap-FGID depending on lifestyle

Growing up in families with a partly anthroposophic lifestyle was associated with a higher prevalence of abdominal pain at five years of age, compared with growing up in a non-anthroposophic family (adjusted OR 2.61; 95 % CI: 1.15-5.93). In the post-hoc analysis we also calculated the corresponding odds ratio for the 125 children in families with *any* anthroposophic lifestyle characteristic, grouping both anthroposophic and partly anthroposophic families together. Growing up in families with any anthroposophic lifestyle was associated with a higher prevalence of abdominal pain at five years of age, compared with growing up in a non-anthroposophic family (adjusted OR 2.50; 95 % CI: 1.17-5.32).

## 5.4 STUDY IV

### 5.4.1 Prevalence of RAP

Prevalence of weekly RAP in early childhood, at 12 years of age and at 16 years of age, were 7 %, 4 % and 20 % respectively. In total, 33 % of all children reported RAP at least once during the follow up. The prevalence of RAP peeked at 16 years of age in both boys and girls. Prevalence estimates were significantly higher for girls compared to boys at 12 and at 16 years of age but not in early childhood.

### 5.4.2 Prevalence of ap-FGID and phenotype distribution

In the cohort at 16 years, 20 % (609/3039) had RAP and out of the 609 adolescents with RAP 498 answered the detailed Rome III questions that enabled classification of ap-FGID subtypes. In 348/498 the Rome III criteria for any ap-FGID were fulfilled. In this subgroup, IBS was found in 6.0 % (175/2926), FAP in 3.4 % (99/2926), FD in 2.7 % (79/2926) and overlapping IBS and FD in 0.2 % (5/2926). The prevalence of ap-FGID was higher in girls than in boys (16 % vs 8 %, $p<0.01$).

### 5.4.3 Turnover

Complaints of RAP over two assessment points was reported in 2.8 % (70/2459) of the children, 77 % of them being girls, and complaints over three assessment points was reported in four children, all girls. Children with RAP in early childhood and at 12 years of age reported persisting symptoms at the following assessment point in 7 % and 43 % of cases respectively. It was more common for girls than for boys to report persisting symptoms between early childhood and 12 years, but not between 12 years and 16 years.

### 5.4.4 Risk of persistent RAP during childhood

There was no statistically significant association between RAP in early childhood and at 12 years of age (adjusted RR 1.7; 95 % CI: 0.9-3.0). (Figure 5). Children with RAP at 12 years of age had an increased risk for RAP at 16 years of age (RR 2.1; 95 % CI: 1.79-2.73) for any ap-FGID at 16 years of age (RR 2.5; 95 % CI: 1.8-3.4) and for IBS at 16 years of age (RR 3.2; 95 % CI: 2.0-5.0). All analyses were adjusted for sex.

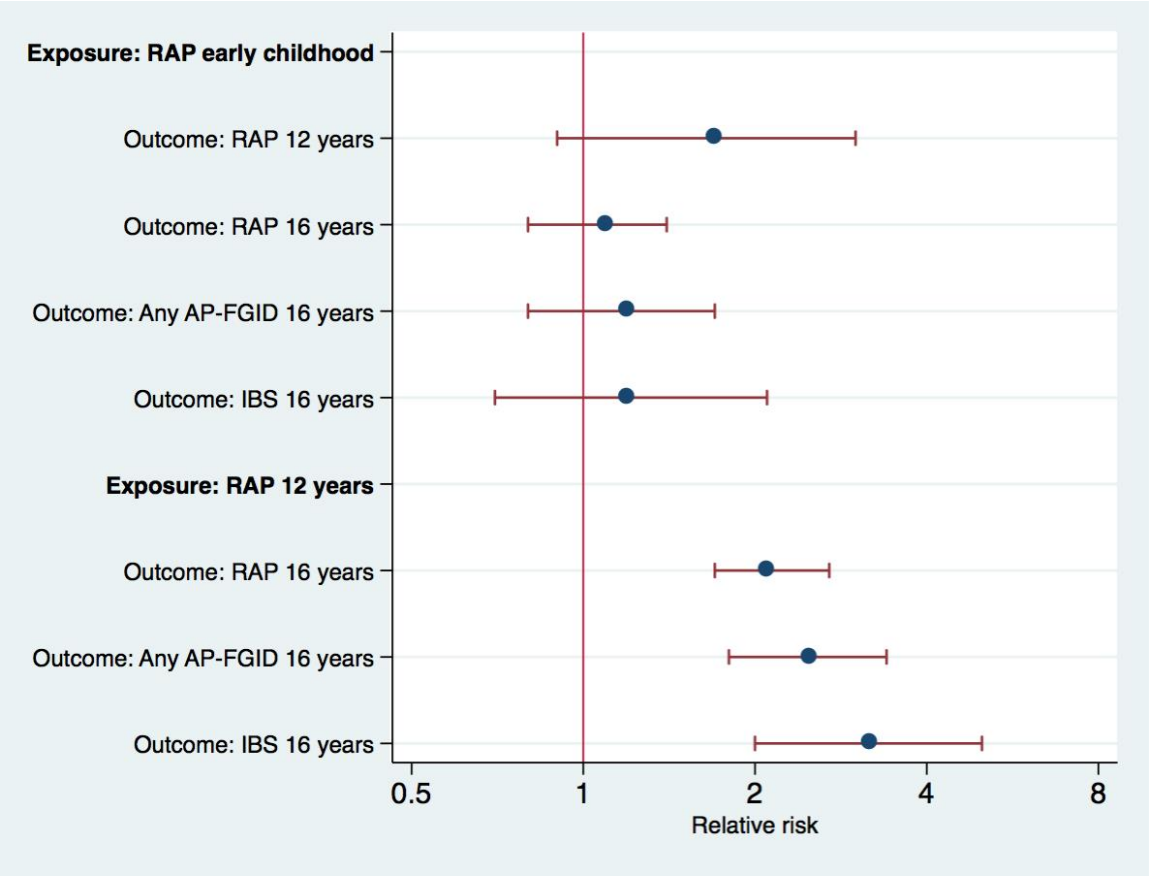


**Figure 5.** Sex-adjusted associations of RAP and Rome III ap-FGIDs and IBS at 16 years by exposure to RAP earlier in childhood in a population-based birth cohort

Total number observations used (listed in the order they appear in the forest plot from top to bottom): 2689; 2927; 2820; 2820; 2524; 2441; 2441

Associations assessed in a binominal generalized linear model with log link

Error bars represent 95% confidence intervals

*Early childhood* includes children 12-24 months of age, and RAP was parent-reported

# 6 ETHICAL CONSIDERATIONS

All studies in this thesis were approved by the Regional Ethics Review board in Stockholm. No pharmacological interventions were performed in any of the studies. In study I, the information about the study was addressed to both parent and child at the doctor's visit, adapted to the child's age and maturity. Written parental consent was required from both when there were two caregivers. Personal data (name, social security number) were kept separate from study data. The principal investigator kept the code keys, but the identity of the study persons was available to the researchers on request.

In the BAMSE and ALADDIN study, parents were recruited and signed informed consent starting in 1994 and 2002 respectively. In follow-up at 12 years in BAMSE, an information letter was sent to the parents with a request to show it to the child. A completed questionnaire was regarded as consent from the 12-year-old adolescent in BAMSE study and at inclusion in study I.

The ethical issues of this research are about asking people questions, about registries and of reading patient records in another purpose than health care. All studies involved parent and /or patient questionnaires, and these could be extensive, requiring time, energy and consideration to fulfil. We must also consider how the questions asked to participants may affect them. Participants may understand what associations we intend to explore, also when we don't express them clearly. We may simply put ideas in somebody's head, by introducing beliefs on false associations, long before the study results are ready.

In the recruitment of patients for study I, my personal experience was that when a patient was a potential study participant and I knew that the record could be reviewed, this could sharpen the consultation. Eventually the patient record writing became more structured, knowing that it could be part of research. It is good for research purposes and for the patient, if the doctor is precise and thorough in the documentation process.

In study III, I made phone calls to parents to confirm questionnaire answers that their child had abdominal pain at least once a week during at least two months. One of the most important ethical issues is that research must not do harm to participants, but in some phone calls I got the impression that my call and questions induced worry, as if the parent got the feeling of having failed to seek health care for the child with abdominal pain. In these situations, I provided more information on ap-FGID and its high prevalence.

# 7 DISCUSSION

## 7.1 DIAGNOSING AP-FGID, STUDY I

In study I we explored if abdominal pain-related functional gastrointestinal disorders (ap-FGID) could be diagnosed with patient- and parent-administered questionnaires based on the Rome III criteria, together with absence of a selection of alarm symptoms. The questionnaire answers regarding Rome III criteria alone could predict ap-FGID with low to moderate accuracy. With addition of alarm symptoms to the questionnaire-based Rome III diagnosis, the specificity for a functional diagnosis was high, but sensitivity was low. If questionnaires based on Rome III-criteria together with absence of alarm symptoms were used clinically, 85% of the children in the study who were finally diagnosed with ap-FGID had needed further investigations. It is doubtful if that would have been ethically advisable. In all children with a symptom profile of ap-FGID according to Rome III and negative alarm symptoms, 17 % would have an organic diagnose according to our study.

The study did not include follow-up questionnaires, so there could be one year between the two time points: 1) when the Rome III questionnaire was answered and 2) when the reference diagnose was set through record review. Symptom development in this time frame is plausible and is often a clinically useful tool. In 2005 a review of 94 articles on chronic abdominal pain in children rated the diagnostic value of alarm symptoms as lowest level of evidence [1]. Some years later the first comparisons and validations of alarm symptoms or red flags in ap-FGID were published [25, 180]. In the update on Rome IV criteria, 13 *potential* alarm features were listed. The note says that '*a clinical judgment should be exercised, putting what might be considered an alarm sign into the whole context of the history and physical examination*'. Between Rome III and Rome IV, the alarm sign 'pain that wakes the child from sleep' has been removed, replaced by '*nocturnal diarrhoea*'. In study I, the alarm sign of nightly awakening due to *pain* was not included but the sign that was asked for: 'waking up because of *defecation* at night' was significantly associated to OGID. Thus, our study seems to have chosen a more valid alarm sign than those postulated in Rome III, resembling the modified alarm sign in Rome IV. A new alarm sign was added in Rome IV, odynophagia, which is specific for eosinophilic esophagitis, an increasing entity in paediatric gastroenterology [181]. We included seven of the 13 potential Rome IV alarm signs and found three of them to be significantly associated with OGID: family history of organic gastrointestinal disease, rectal bleeding and defecation at night. In summary, alarm symptoms for organic disease in children with ap-FGID is a field with scarce knowledge and a large need for more validation studies.

Study I is an observational study with prospectively collected data. A major strength is the detailed description of performed analyses and investigations in children seeking care for potential ap-FGID, and the long follow-up in records of previous health-care contacts in children with functional gastrointestinal disorders (FGID). This was possible thanks to the digital patient records.

The limitations of study I depend on factors often associated with studies performed in clinical settings. We had to balance between power (best possible participant recruitment) and strict inclusion criteria. We excluded 80 participants who had a FGID (and not an ap-FGID) but in the validation analyses inclusion or exclusion of these 80 participants hardly affected the results. To enable inclusion at different sites and by many different nurses and doctors, we decided to ask patients for participation when clinically possible. This may have introduced a selection of participants. Children / adolescents with long and complicated complaints may not have been invited because the staff thought it cumbersome for a family already in great suffering. According to the referral routines at the Hospital in question, patients with a high suspicion of inflammatory bowel disease (IBD) or celiac disease (CD) are directed for endoscopy without a previous outpatient visit. In the actual sample the number of children with OGID is lower than expected, which may have decreased the generalisability of the study. Generalisability could have been improved if all children with a consultation because of gastrointestinal complaints at the recruiting centres had been included, and if those who declined to participate had been possible to characterise and compare to those included.

## 7.2 RISK FACTORS

STUDY II and III explores risk factors for ap-FGID in childhood, using two different birth cohorts. Research on risk factors of a disorder is especially interesting when the association is strong, and you can minimise the risk of disease by eliminating the risk factors but identifying risk factors can also lead to important clues about disease and pathogenic mechanisms. When mechanisms are elucidated, the possibilities of developing effective treatment are improved.

### 7.2.1 Antibiotic treatment, Study II

In study II we investigated antibiotic exposure in relation to RAP at 12 years of age. We did not find a statistically significant associations between antibiotic use and monthly RAP at 12 years. The large, population based, prospective BAMSE cohort with small loss-to-follow-up is a major strength of study II. Insufficient statistical power may always introduce a risk of not detecting associations. This possibility must be considered also in large studies like this one. Thus, we cannot exclude that a larger sample size would have found an association between antibiotic treatment and RAP. For instance, we found that three or more courses of antibiotics were almost significantly associated with a 50 % higher prevalence of monthly RAP (OR 1.54, 95 % CI: 0.99-2.49). Moreover, in the subgroup of 40 children who were exposed to tetracycline treatment, six children had RAP at 12 years and the OR of monthly RAP at 12 years was 1.80 (95 % CI: 0.74-4.34).

The Swedish Prescribed Drug Register is a highly reliable source with the limitation that it did not start until 2005, and that it registers dispensed drugs, not used drugs [182].

It is a limitation that the design of the questionnaires did not allow us to use definitions according to Rome III criteria at 12 years. The definition of RAP in Study II applied to

Apley's with *monthly* abdominal pain. By adding post-hoc analysis of *weekly* abdominal pain, the comparability to Rome III was made easier. No major differences in the risk of RAP were revealed with the outcome of weekly RAP.

Since a parental risk factor for children's RAP is anxiety, this trait might increase the probability of a doctor's visits of any cause. Consequently, children with an anxious parent may seek a doctor more often, and a doctor's visit per se may hypothetically increase the risk of antibiotic treatment. Theoretically, children with increased risk of RAP may have received more antibiotics, thereby introducing a kind of confounding by indication. This could theoretically have increased the ORs in the study. The exclusion of 20 children with CD and IBD in Study II could be questioned, since these participants were not excluded in Study IV, but there classified as non-RAP. At least IBD patients should stay excluded in Study II, since they are often treated with antibiotics and would introduce differential misclassification. CD patients could have been kept but due to the small number it would probably not have affected the result.

Due to the population-based design and relatively high follow-up rates, the generalisability of results is deemed high.

### 7.2.2 Lifestyle, Study III

Lifestyle characteristics as risk factors were explored in Study III. No single characteristic of the anthroposophic lifestyle could be identified as a risk factor but taken together, this lifestyle was associated with an increased risk of ap-FGID at five years of age. Several possible factors of the anthroposophic lifestyle were candidates for this increased risk of ap-FGID. For example, consumption of fermented food and less antibiotic use thereby modifying microbiota, and a vegetarian diet through a higher fibre content. Starting pre-school at an older age could affect several psychological factors including coping strategies. Having two or more older siblings was more common in anthroposophic families but also independently associated to ap-FGID. Sibling rivalry was predictive of non-organic cause of abdominal pain in an Indian study but having older siblings has not been studied [183]. Inversely, Alfvén found jealousy towards younger sibling in two cases of psychosomatic abdominal pain [111].

Previous research on the influence of socioeconomic factors and educational level on ap-FGID is contradictory. In paediatric studies economic stress and lower socioeconomic stability, has been associated to an increased risk of ap-FGID, while adult irritable bowel syndrome (IBS) has been linked to a higher education and childhood affluence [87, 88, 184, 185]. A recent meta-analysis showed no relation between IBS prevalence and national socioeconomic status, but there are no similar surveys on the wider concept of ap-FGID [186]. A plausible explanation of the two-fold increased risk of ap-FGID in five-year olds of anthroposophic families is the impact of exposure to (adverse) life events. Psychological research indicates that a moderate number of adverse events are beneficial, when compared to a history of none or repeated adverse events [187-189]. A characteristic of the anthroposophic lifestyle is parental

ambitions to protect their children from unpleasant external stressors, and this is suggestive to account for the lower evening salivary cortisol levels in their offspring [97 190]. Differences between anthroposophic versus non-anthroposophic parents concerning stress management are probably most pronounced in everyday life. For example, variations in the number of daily activities and new situations the infant meets in early life, at what age they start day-care and for how many hours. We hypothesize that infants of anthroposophic families are less exposed to new or strange situations, thereby getting less exercise in coping with what is new and different, for example sensations from the gastrointestinal canal. The generation R study performed Strange Situation Procedures in 14-month-old infants, to assess their ability to cope with stress and linked it to salivary cortisol levels [191]. Cortisol stress reactivity when left alone with a stranger was slightly, but not significantly higher in infants with abdominal pain than in those without. Another very important experiment has been performed on children 8-16 years of age with RAP and healthy controls[192]. Parents were randomly assigned three different instructions on how to deal and react to their child's experimentally induced pain: attention, distraction, or no instruction. Both in RAP-patients and well children parental distraction gave the lowest pain scores in response to the experimental pain [192]. Parent attention reinforced the child's pain in both groups, but the effect size was larger in pain patients and more pronounced in females.

A strength of study III is that the definition of ap-FGID adheres to Rome III criteria and that children with constipation or minor abdominal pain could be identified. The relatively small sample size entailed a risk of type II-error, but nevertheless we could detect a significantly increased risk of ap-FGID in children of anthroposophic families.

A limitation is the lack of information on parental ap-FGID since it might be a potential confounder. Genetic or social trait in ap-FGID has been explored, but only in a few studies [39-41]. The children of our study may have had an increased hereditary risk of ap-FGID. Parents who choose to live according to the anthroposophic lifestyle could have chosen it due to susceptibility to stress and even be more prone to disorders where stress is thought to be involved, like IBS. If a hypothetical scenario with a higher prevalence of parental ap-FGID is correct, the increased risk we found in children of these families could be attributed to selection bias. Another possible but opposite scenario is that these parents have improved skills in dealing with environmental stress, and that their children might have displayed an even higher prevalence of ap-FGID without their parents' capacity in stress coping.

The families in the ALADDIN cohort were considered representative of the residential area. The non-anthroposophic families are considered representative of the general population since prevalence of traditional risk factors of allergy was similar to the larger BAMSE study [193]. The generalisability is therefore regarded as reasonably high.

## 7.3 PROGNOSIS, STUDY IV

In study IV the prognosis of RAP was explored, from the age of 12 months to 16 years of age. RAP is a chronic disorder with an intermittent character. The prognosis is important for the patient, the parents and the doctor. Two large, population-based, longitudinal, prospective birth cohort studies on prognosis have been performed with 29 years and 26 years of follow-up, respectively [8, 194]. British children with persistent RAP on three measurements in childhood, at 7, 11 and 15 years were evaluated for physical symptoms and psychiatric disorders at age 36 years. The main finding was that the children with RAP in childhood had a doubled risk of an emotional disorder, and no increased risk of a physical disorder when controlling for psychiatric disease [8]. In the Australian study, a moderately increased risk of adult IBS, defined according to Manning, was observed in adults who had a history of RAP at 7-9 years of age [194].

The association between infant colic and RAP is not well explored. Canivet found that ex-colicky children displayed more negative emotions and stomach pain at 4 years of age, although stomach pain was not strictly defined [195]. Ramchandani et. al. found a considerably increased risk of persistence from two years of age up to school-age [196]. This finding of persistent RAP was not confirmed in study IV. Other studies have focused on influence of RAP-associated symptoms, like headache, limb ache and back-pain. For example, Walker et. al. showed that children with ap-FGID in combination with non-abdominal pain(s) had a doubled risk of persistent ap-FGID 10 years later [197]. In a review by Gieteling et. al., parental gastrointestinal problems were a risk factor for RAP persistence [114].

The strengths of study IV are similar to study II, namely the large BAMSE cohort and an even better follow-up rate at 16 years than at 12 years plus the Rome III defined ap-FGID at 16 years. As in all questionnaire studies, there is a risk of recall bias. Parents answering questions on RAP in the preceding 6 months when child is 12 and 24 months probably have a better memory if RAP was difficult and long-lasting, which will underestimate the prevalence at these ages.

## 7.4 GENERAL DISCUSSION

This thesis is based on four prospective studies aimed to elucidate the large clinical entity of RAP and ap-FGID in childhood. Recurrent abdominal pain in children is recognized in science as a frequent and troublesome disorder in childhood since more than 60 years [11, 198]. The current perspective is biopsychosocial, and the cause is regarded multifactorial.

This thesis showed that one third of Swedish children between one and 16 years experiences RAP. In an individual perspective, i.e. the parent who seeks health-care for their child, the most important is to exclude that the child has an organic disease. In a social and economic perspective, it appears equally essential to improve diagnostics and treatment of children with ap-FGID. The future dealing with the pain in the family is influenced by many factors. It can

be hypothesised that one factor is the quality of the consultation. Were the family's questions addressed? Medical consultations are improved by asking about the patient's expectations, fears and imaginations about disease, and most likely this is true also in the context of ap-FGID [199]. Anxiety in parent and child is associated to RAP. Abdominal pain and anxiety can both be serious and debilitating conditions and must be taken seriously when dealing with this group of patients. Consultation and trust seems to be the way to success, where repeated visits to the same doctor is a useful tool [5].

Chrushell et. al. has shown that the prognosis is poorer for a child with RAP whose parents consider/believe in an organic origin of pain[200]. Parents' beliefs are probably affected by several factors both within health-care and outside the control of doctors and other professionals. RAP in children can be chronic, very often relapsing and in a group of children, regardless what actions are taken, there will be repeated doctor's visits over many years.

The prognosis study showed that the general prognosis of RAP is good, and that most children with RAP grow out of it. All the same, having RAP increases the risk of future RAP, ap-FGID and IBS. The knowledge that RAP usually is a transient disorder is a very important message for paediatricians and generalists to worried children and parents.

# 8 CONCLUSIONS

- A Rome III-based patient-administered questionnaire to children seeking care for gastrointestinal symptoms had low to moderate sensitivity, specificity and positive predictive value in predicting an ap-FGID diagnosis, based on a record review one year after the questionnaire was filled in
- When adding a prerequisite of negative alarm symptoms to the Rome III questionnaires, specificity became high but due to low sensitivty, very few children could be diagnosed with ap-FGID
- Antibiotic treatment in childhood was not a major risk factor for RAP
- Children growing up in a family with an anthroposophically inspired lifestyle had an increased risk of IBS, FD and FAP at five years of age
- Most children with RAP in early childhood or at 12 years of age do not have persistent paediatric RAP
- RAP at 12 years was an independent risk factor for RAP at 16 years of age
- One of three children had RAP at least once between one and 16 years of age

# 9 FUTURE PERSPECTIVES

The international research in paediatric FGID is extensive but not in relation to its prevalence. Probably, studies on psychological and dietary treatment will increase.

Other targets that would be interesting to address is the doctor's worries in managing children with RAP. If the doctor is worried it will remain hard to comfort the family. Could this be altered by team-work and peer mentorship?

Alarm symptoms in ap-FGID need more scientific attention. It would be interesting to study if a selection of suggested alarm symptoms could improve diagnostic efficacy, certainly if asked and evaluated by the doctor and not in a patient-administered questionnaire. Further, it would be interesting to study health care seeking, and if children with ap-FGID and their parents who seek medical advice are different from those who do not. Children with ap-FGID who seek health care, do they have more alarm symptoms than children with ap-FGID not seeking health care?

# 10 SAMMANFATTNING PÅ SVENSKA

Det är mycket vanligt att barn har återkommande magont (eng: Recurrent Abdominal Pain RAP). Den polade prevalensen i studier från hela världen är 13,5 %. Med återkommande menas att barnet besväras regelbundet under minst 2–3 månaders tid. Orsakerna är många och kan vara organiska/medicinska eller funktionella. Exempel på medicinska orsaker är inflammatorisk tarmsjukdom och celiaki. De funktionella orsakerna definieras utifrån symptom. Det finns ingen provtagning som kan bekräfta att RAP beror på funktionell magtarmsjukdom (FMT). FMT definieras för både barn och vuxna enligt internationellt accepterade kriterier, de s.k. Rom-kriterierna. Rom-kriterierna för barn kom 1999 och har uppdaterats 2006 och 2016 och indelas i smärt-dominerade och icke-smärtdominerade FMT. De smärtdominerade FMT enligt Rom III innebär magont minst en gång i veckan under minst två månader i följd. Irritabel tarm, funktionell dyspepsi och ospecificerad funktionell buksmärta är de tre smärtdominerade FMT. Bukmigrän hör till denna grupp men har annorlunda frekvens och duration och undersöks inte i denna avhandling.

Orsaken till funktionell magtarmsjukdom betraktas som multifaktoriell, dvs orsakerna är många och sammansatta. De bidragande faktorer som forskning kunnat identifiera är associerade med ärftlighet, tarmfloran, inflammation, förändrad tarmrörlighet och smärtupplevelse, kost samt psykologiska faktorer som traumatiska upplevelser, depression och ångest. ”Gut brain-axis” är ett centralt begrepp, och innebär att smärtan vid funktionell magtarmsjukdom anses bero på ett samspel mellan hjärnan och magtarmkanalen. Tarmen är försedd med ett utbrett nervsystem och av ett immunologiskt aktivt system. Interaktionen anses medföra att tarm och hjärna påverkar varandra i båda riktningar.

Möjligheten till läkemedelsbehandling vid FMT är ytterst begränsad. Ett biopsykosocialt synsätt anses mest framgångsrikt vid behandling av FMT hos barn. Det betyder att man vid behandling behöver ta hänsyn till både biologiska system, psykologiska och sociala faktorer. Patient-läkar-relationen är av stor betydelse när det gäller information och lugnande besked till patient och föräldrar. Det pågår mycket forskning inom psykologisk behandling, t.ex. kognitiv beteendeterapi och i bland annat Nederländerna har man studerat hypnos till barn med FMT med goda resultat. Försök med kostbehandling syftar oftast till att minska intag av jäsningsbara kolhydrater, vilka anses kunna bidra till smärta vid FMT. Det pågår sådana studier hos barn, men det är viktigt att de är dubbel-blint utförda med placebo-kontroll. Vid kostbehandling till barn är det väsentligt att försäkra sig om att det inte försämrar intaget av energi och näringsämnen för en växande individ.

Syftet med denna avhandling var att kartlägga riskfaktorer för FMT hos barn, att studera hur tillgängliga diagnosmetoder fungerar och att studera prognosen för barn med FMT.

Denna avhandling bidrar till ökad kunskap om FMT hos barn genom dessa fynd:

- De diagnosmetoder som finns tillgängliga genom frågeformulär från Rom-kommitten är måttligt bra på att ställa diagnosen FMT. Det innebär att om dagens diagnosformulär och alarmsymptom användes i diagnostiken skulle 85 % av barnen med FMT vara tvungna att genomgå ytterligare kanske smärtsamma procedurer utan att det ger någon mer användbar information
- Dessa frågeformulär är bra på att utesluta att magont inte beror på organisk sjukdom, men bara hos en liten andel av de som har återkommande magont
- Antibiotikabehandling, som anses vara en riskfaktor för vuxna med FMT, ökade inte risken för FMT hos barn som behandlats
- Barn i familjer med en delvis antroposofisk livsstil hade en ökad risk för FMT vid fem års ålder
- Den antroposofiska livsstilen innehåller flera faktorer som skulle kunna förklara denna skillnad, men vi fann ingen enskild faktor i vår studie som stod för riskökningen
- Barn som har återkommande magont (RAP) vid två respektive 12 års ålder har det oftast inte vid uppföljning vid 12 eller 16 års ålder. RAP vid 12 år är ändå en riskfaktor för RAP vid 16 år
- Cirka en tredjedel av svenska barn har återkommande magont någon gång mellan ett och 16 års ålder

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